"THE BLOOD OF RUMINANTS AND THE HAEMATOLOGY OF THE DOMESTIC FOWL IN HEALTH AND DISEASE".

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PREFACE

The work associated with this thesis commenced with the writer's tenure of the James Tindal post-graduate Scholarship, 1928-29, Royal (Dick) Veterinary College, Edinburgh, under the supervision of Sir Edward Sharpey Schafer. From the Department of Anatomy at this College, it was continued at the Poultry Diseases Diagnosis Department, Veterinary Laboratory, Ministry of Agriculture and Fisheries, Weybridge, Surrey (1931), and has been finished at the Veterinary Laboratory, School of Agriculture, Plumpton, Sussex, (1936-38).

An association with Dr. Henry Dryerre and Dr. Eric Ponder of the Physiology Department, The University, Edinburgh, first gave the writer an insight into the study of blood cells, particularly the latter's work on the modified "Arneth" count.

As Centenary Research Fellow (Edinburgh) 1929-30, visits were paid to Professors Schilling, Hirschfeld and Arneth in Germany, where facilities were provided for studying the methods employed in routine blood work.

Both before and after these Continental visits, short periods were spent working in the pathological laboratory at The Cancer Hospital (Free), London, where, thanks to the generous tuition of Dr. Alfred Piney, a sound introduction to the subject of clinical haematology was learnt and the associated technique acquired.

INTRODUCTION

When this study was commenced in 1929, examinations of bovine or other ruminants' blood were of insignificant importance clinically, and poultry played no part in the life of the average veterinarian. Although there has been little change with regard to the applications of haematology in cattle practice, the heavy mortality of poultry, particularly with reference to "fowl paralysis" and associated diseases, has resulted in the establishment of veterinary laboratories where examinations of poultry post-mortem have been carried out extensively.

During the past eight years, the writer has had valuable opportunities to investigate outbreaks of poultry disease, both in the field and laboratory, and to carry out additional haematological examinations of bovines. This thesis embodies the results of these observations and blood cell examinations.

In order to become familiar with the blood cells of man, and prior to the examination of the blood cells of animals, a short study was made of the blood of normal veterinary students (Blount 1935), and also of certain hospital patients suffering from pernicious anaemia, myelogenous and lymphatic leukaemia.

Although there are now a considerable number of publications dealing either with the blood of the domestic hen or of cattle and sheep, the original references were few, of which two of the best were Burnetts monograph "The Clinical Pathology of the Blood of Domesticated Animals," and Gullard and Goodall's textbook "The Blood." Dr. A.C.Fraser's excellent treatise on "The Blood of Cattle and Sheep in Health and Disease," although published in 1930, has only recently been read by the writer. It has proved valuable as a basis for discussion, and for comparison with certain of the results of the present investigation.

Full use has been made of a number of haematological textbooks - a list of which is appended later in the thesis.

OBJECTS OF THE INVESTIGATION

The general objects of the investigation were:-

(1) To study the histological appearances of the cells concerned.

(2) To determine whether normal blood standards, comparable with those of man, exist in the species examined.

(3) To observe the changes occurring in the type and distribution of blood cells following birth.

(4) To ascertain whether the differential count is applicable to cattle or poultry as an aid to diagnosis.

(5) To record the blood pictures occurring in the course of certain diseases, and

(6) To find suitable subjects for further research studies.

While the above represent the general objects to be attained, the special study was one of purely veterinary importance, i.e. of endeavouring to find a technique suitable for field investigations, and capable of being applied and interpreted by the average veterinary practitioner. In conjunction with this, it was also necessary to decide which method of expressing the blood picture would be most appropriate from a clinical point of view. The methods and counts employed by the veterinary pathologist are not necessarily those of the practitioner on the farm, whose sole object in taking blood smears is to acquire the information they can give as rapidly as possible, and apply it for diagnostic or prognostic purposes.

Briefly, therefore, the work has resolved itself into a study of the practical applications of clinical veterinary haematology.

GLOSSARY

Unfortunately, the terminology generally used in connection with the examination of mammalian blood cells is unsuitable in certain respects for birds. This is especially true in a study of avian erythrocytopoiesis, and with reference to certain of the granulocytes. Complex terms, with little appreciation of the functions of the cells concerned, have been invented. Many of them are confusing, and therefore a short glossary of selected terms follows, and where possible, strict adherence to recognised nomenclature (relative to form and function) has been practised.

"ADULT" POLYMORPH - A neutrophile with a complex nucleus - usually polylobular.

ANISOCYTOSIS - Marked variation in cell size - notably of the erythrocytes.

ARNETH COUNT - The specific blood count of Arneth (1904) involving the classification of leucocytes according to their nuclear complexity.

AZUR "BODIES" - Specific cherry red granules or masses found in certain mononuclears after staining with Giemsa compounds.

"BAND" - One of Schilling's specific metamyelocytes, with deep though simple indentation of the nucleus. Synonym: Stabkernige.

BASIPHILE - Synonym: basophile.

"BUDDING" - A characteristic clumping of the cytoplasm of mononuclear cells leading to cell distortion.

CORPUSCLE - A non-nucleated progeny of a cell.

ERYTHROBLAST - A myeloid precursor of the erythrocyte.

ERYTHROCYTE - Normal mammalian red corpuscle; normal avian red cell.

ERYTHROPLASTID - A non-nucleated derivative of an erythrocyte - an avian red corpuscle.

FUSIFORM CELL (McGowan) - The avian thrombocyte.

GRANULE-BEARING EOSINOPHILIC POLYMORPHONUCLEAR - The avian eosinophilic granulocyte. Synonym: eosinophile.

GRANULOCYTE - Any white blood cell normally having a full complement of granules.

HAEMOCYTOBLAST - The undifferentiated stem cell - considered by Emmel to be an immature lymphocyte.

HAEMOGLOBINIFEROUS ELEMENT - Any structure capable of transporting haemoglobin in the blood circulation.

IMMATURE LYMPHOCYTE - Emmel's "haemocytoblast."

LEUCOCYTE - Any white blood cell.

LYMPHOCYTE - A non-granular leucocyte with a characteristic disposition of its oxy- and basi-nuclear chromatin. The cytoplasm sometimes contains azur bodies.

MEGALOBLAST - The large nucleated precursor of the megalocyte.

MEGALOCYTE - The fully haemoglobiniferous derivative of the megaloblast - frequently hyperchromatic and whose average size is greater than that of the normocyte.

METAMYELOCYTE - A stage in the development of the granulocyte between that of the myelocyte and "adult" types.

MONOCYTE - The largest circulating agranulocyte with characteristic nucleus of linear (not "blobby") appearance. Derived from the reticulo-endothelial system.

MONONUCLEAR - Any non-granular white blood cell with a monolobular nucleus.

MYELOCYTES - Granulocytes with rounded nuclei and usually an incomplete complement of granules.

NUCLEAR APPENDAGE - An abortive nuclear lobe attached to a main lobe - notably in the mammalian polymorph.

NUCLEAR "NOTCH" - An obvious indentation of the periphery of the nucleus of an avian erythrocyte.

NUCLEAR REMNANTS - Referable to the deformed degenerate nuclei seen in the blood-stream after intravascular haemolysis of avian red cells.

NUCLEOLUS - (1) Karyosome - an aggregation of deeply basic nuclear material.
(2) Plasmosome - an oval light staining area in a nucleus usually clearly circumscribed and considered indicative of youth.

NEUTROPHILE - Synonyms: neutrophilic granulocyte; "polymorph;" polymorphonuclear neutrophilic leucocyte.

NORMOCYTE - Normal circulating RBC, mammalian or avian. Synonym: erythrocyte.

"PLASTID" FORMATION - The formation of red blood corpuscles from erythroblasts by karyolysis (mammalian) or nuclear extrusion (avian).

POIKILOCYTOSIS - Cell distortion - usually referable to pear-shaped erythrocytes.

POLYCHROMASIA - A peculiar staining of the cytoplasm of red blood cells associated with an incomplete complement of haemoglobin.

POLYCHROME ERYTHROCYTE - An immature normocyte without its full complement of haemoglobin.

POLYMORPH - The polymorphonuclear neutrophilic granulocyte.

POLYNUCLEAR COUNT - The specific blood count of Cooke and Ponder (1927) designed to assess the nuclear morphology of the neutrophile.

PRE-GRANULAR BASIPHILE MYELOCYTE (AVIAN) - A basiphile granulocyte with round nucleus, but containing few or no true granules.

PRIMARY ERYTHROBLAST - The basic non-haemoglobiniferous stem cell of the erythrocyte series.

PSEUDO-EOSINOPHILE - The avian neutrophilic granulocyte.

PUNCTATE BASIPHILIA - Synonyms: P. basophilia; stippling.

PYKNOSIS - A condensation of parts leading to a strong affinity for basic stains - usually of the nucleus.

RED BLOOD CORPUSCLE - The normal, circulating, non-nucleated haemoglobiniferous element in mammalian blood; Synonym: erythroplastid.

REIDER CELL - A monocyte or lymphocyte showing a cleft nucleus.

RETICULOSIS - increase of polychromasic (reticulocytes) normocytes in the general circulation.

ROD-BEARING EOSINOPHILIC POLYMORPHONUCLEAR - The avian neutrophilic granulocyte.

SCHILLING COUNT - A specific method for classifying the granulocytes based on nuclear morphology.

SHIFT TO THE LEFT - Indicative of an increase in the number of circulating immature granulocytes.

SHIFT TO THE RIGHT - Associated with the presence of an excess number of hypersegmented granulocytes.

THROMBOCYTE - A specific nucleated avian blood cell characterised by its vacuolated cytoplasm and inclusion granules. It is believed to be homologous, in function, with the mammalian blood platelet.

TOXIC GRANULES - Usually of the neutrophile - depicted by clumping and pyknosis of the neutrophilic granules.

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TURK CELL - A monocyte, lymphocyte or allied cell with deeply staining (basic) cytoplasm.

VACUOLAR LYMPHOCYTE - Emmel's description of the avian thrombocyte.

VACUOLATION - The presence of vacuoles.

WEIGHTED MEAN - The specific index of Ponder and Flinn (1926) introduced to simplify the expression of the polynuclear count in terms of the average number of nuclear lobes per cell.

"YOUNG" - A metamyelocyte with simple indentation of the nucleus; Synonym: Jugendliche.

It will be realised that the term polymorphonuclear neutrophilic granulocyte is an unsatisfactory one if the cell concerned has its nuclear morphology normally hidden by a full complement of "pseudo-eosinophilic rods." Yet the avian leucocyte physiologically comparable with the mammalian neutrophile, is such a cell, and has been referred to in some veterinary literature as the "rod-bearing-pseudo-eosinophile." However, since the function of these two cells is apparently identical there seems little objection to the use of the term neutrophile, especially when the eosinophile of the domestic hen in itself is quite distinctive histologically.

Curiously enough, "normocyte" is a more appropriate term for the avian red cell than the mammalian erythrocyte which, as is generally appreciated, is non-nucleated and therefore a red corpuscle. The American term "erythroplastid" though technically correct is cumbersome, and is better suited for those special red cells seen in erythroblastosis foetalis of the chick, and which have lost their nuclei by extrusion <u>in toto.</u>

Again, with reference to the red cells, the terms megaloblast and megalocyte are used by various authors in a different sense, for few agree with Piney that megaloblasts and their progeny represent a distinct line of erythropoiesis. However, since the present studies have shown clearly that certain circulating red corpuscles in the bovine are both hyperchromatic (fully haemoglobiniferous) and larger than the average normocyte, the term megalocyte has been restricted to them.

On the recommendation of Sir Edward Sharpey Schafer the common term "basophile" has not been used by the writer of this thesis, basiphilic - referable to the affinity for basic dyes - having been substituted.

From a study of the high percentages of monocytes recorded for normal bloods by some writers, it is clear the distinction between the true monocyte (the large hyaline leucocyte) and the large lymphocyte has not been appreciated fully. The writer has followed those workers who consider the difference lies essentially in the histological character of the nucleus, rather than on the actual size or relative sizes of the cells concerned. Even so, some difficulty has been experienced with the blood of cattle, deciding to which class certain of the mononuclears belong - a difficulty readily appreciated by Fraser and others who have also studied bovine blood cells critically.

TECHNIQUE

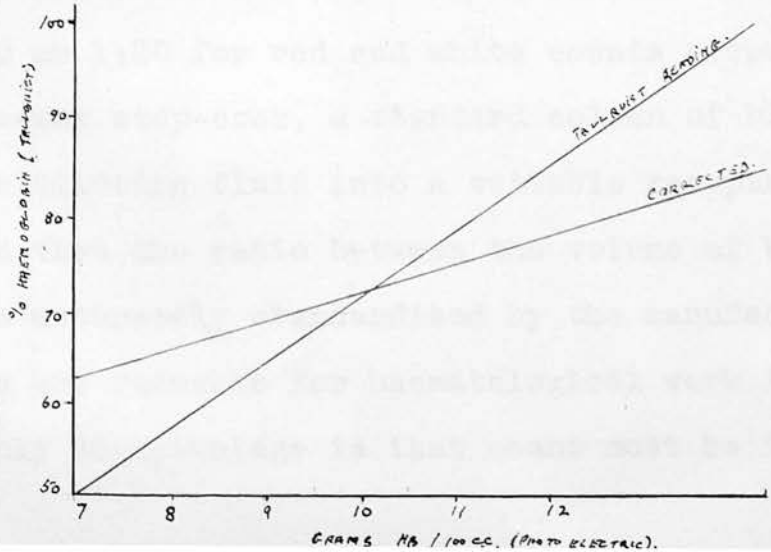
Source of Blood

As a routine practice, samples were taken from one of the superficial ear veins in bovines, and from the wing vein (V.cubiti medialis) in poultry. On a few occasions, it was found more convenient to take samples from the mammary vein in cattle, and from the jugular vein in young chicks. A venous source of blood for cell examination purposes is probably superior to that usually used in man, i.e. the ear lobe or tip of the finger, since these latter sites yield samples of capillary blood sometimes possibly contaminated by lymph cells. In all cases, a sharp, pointed, triangular "pricker" needle was used to puncture the vein, similar to that used for "blood testing" purposes relative to the B.W.D. agglutination test. Following the removal of hairs or feathers, the skin was cleansed when necessary with a swab of cotton wool and chloroform, which had the additional value in poultry of causing the superficial veins to dilate.

Tests were carried out to ensure that the results obtained were satisfactory, and provided that a routine procedure is adhered to, and a free flowing venous supply of blood available, the readings appear to be reasonably constant, and well within the limits of experimental error. Full details of these tests are recorded later in the paper, (page 29).

Haemoglobin Estimations

The Dare and Tallquist methods have both been employed. Although the Tallquist tinted scale is generally considered unsatisfactory for accurate experimental work, if care is taken to examine the blood immediately it is dry, and provided that the common error of giving readings which are too high is avoided, i.e. by always working upwards on the scale (e.g. from 50-70-90) this instrument can prove of great value in veterinary practice. Olson (1935) has plotted carefully the corrections necessary for adjusting the % figures obtained for poultry blood by the Dare and Tallquist methods into grammes of haemoglobin. Similarly, Dukes (1937) has emphasised the importance of workers realising that results using one haemoglobinometer will differ from another, due to the variations in the colour standards used relative to the actual amount of haemaglobin in the blood concerned. A chart embodying these points follows herewith, and has been used as a basis throughout the thesis.



Note

Dukes and Schwarte (1931) have demonstrated that the use of the Newcomer instrument for the estimation of haemoglobin in the fowl is limited, because of a turbidity developing due to the presence of nucleated cells. Olson (1935) also confirms that "this instrument is not applicable for chickens ill with leukosis as the increased number of immature cells increases the turbidity of the acid haematin solution and disturbs the reading of the sample." Olson favours the photo-electric haemoglobinometer for use on poultry, but no opportunity has occurred for the present writer to utilise this method, and in view of Dukes and Schwarte's conclusion, no attempt has been made to use the associated Sahli method.

Total Red and White Blood Counts

For blood dilution purposes, the writer has used automatic diluting pipettes. These were invented by Dr. Hirschfeld of Berlin, and have been popularised in this country by Piney and others. The usual dilutions are 1:100 or 1:200, and 1:10 or 1:20 for red and white counts respectively. By means of a two-way stop-cock, a standard column of blood is blown out with the diluting fluid into a suitable receptacle or "blood pot." Provided that the ratio between the volume of blood and diluting fluid is accurately standardised by the manufacturers, these pipettes are valuable for haematological work in the field. Their only disadvantage is that means must be found for washing

out the unused blood from the pipettes immediately after use.

The diluting fluids used have been as follows:-

For red counts (a) normal saline solution, or (b) a physiological saline solution prepared by using Parke Davis' glucose saline tablets - the formula for which was suggested to the writer by Professor Henry Dryerre. They have been used successfully in veterinary practice in the control of acute gastritis in dogs, and for the treatment of specific canine hysteria - a note concerning them being published in the Veterinary Record - Blount, 1930.

For the dilution and enumeration of white blood cells, 4% acetic acid has been used for bovine bloods, and distilled water for leucocyte counts in poultry.

An all-glass Bürker haemocytometer has been used with improved Neubauer ruling, the double chamber proving useful for the rapid counting of red and white cells. For each estimation, the cells within 100 squares have been counted in the usual manner.

Total Leucocyte Counts in Avian Bloods

The literature concerning this subject is interesting. Realising that the first essential for a successful total white cell count for mammalian blood is the rapid destruction of the red corpuscles by lysis, investigators at once appreciated that such a method could not succeed with avian blood, because both the red and white elements are nucleated cells. Indeed, as recently as 1926, McGowan writes "Estimation of the number of

leucocytes in normal fowls blood can be done easily when the red blood cells are being counted in the Thoma-Zeiss chamber, owing to the red cells being oval, and the leucocytes round. Because of the abnormal shapes, however, which the erythrocytes assume - round forms, etc. - in anaemic conditions, the same method is not available in such circumstances. As an alternative it has been suggested that every blood cell, red and white, should be counted in the Thoma-Zeiss chamber. Subsequently a differentiation could be made on a stained film, and the absolute number of each obtained in this way. In birds blood, however, there is a great tendency to rapid clotting and for leucocytes and Fusiform cells to run together in heaps at the end of the film. Because of this, enumerations by means of this method can only be approximate, if not actually misleading, in many cases. In the present investigation, no attempt has therefore been made to obtain a numerical expression for the number of leucocytes in diseased fowls blood."

Therefore, for some time laboratory workers were content to use indirect methods, but Blain published in 1928 an account of a new and successful method employing a neutral red diluent which stained the white cells, leaving the erythrocytes clearly distinguishable. Although not difficult, the technique is rather fastidious, since it requires two solutions (adjusted to pH 7.4) to be kept at a temperature of 39°C. while in use. Further, the

method requires the use of a Thoma-type pipette - those of the automatic type having to be discarded. Forkner (1929) used a similar diluting fluid containing neutral red, whereas Doan, Cunningham and Sabin (1925) and later, Keyes (1929) recommended the use of 2% osmic acid. Shaw's direct method (1930) was even more complicated, because the neutral red and crystal violet solutions require to be filtered and heated to 107°F. for use, and in this method also, automatic pipettes are useless. Wiseman (1931) suggested the use of a diluent containing the dye phloxine which stains the eosinophile and neutrophil granulocytes - the total leucocyte count being calculated from the percentage of these cells in a differential count made from the same sample of blood. However, to obtain the optimum staining effect, Wiseman recommends that the filled pipette shall be allowed to stand several hours, which renders the method somewhat impracticable for the clinician.

In a comparative study of the subject, Olson (1935) found that for direct counting methods, those employing Toissons solution were the most satisfactory, although unfortunately in making his differential counts only 100 leucocytes were classified. It is generally accepted, and will be confirmed later in this thesis, that 300 white blood cells is the minimum number to be examined for a satisfactory differential count. A further criticism of Olson's paper is that although "all chickens were apparently healthy and normal" their total white cell counts were respectively (Toisson method) 10,900; 69,400; 23,300; 45,500 and

70,500. From the statement just quoted, it is apparent that Olson considered the birds healthy and suitable for such an experiment, yet no confirmatory post-mortem examinations of the birds were made, and from the leucocyte counts given, three if not four of the five chicken were abnormal, if not definitely pathological. (In a footnote Olson states that he considered the fifth chicken to have been suffering from erythroleukosis).

It will be appreciated that efforts directed to achieve a satisfactory staining method using the 1/6" objective and a specially adjusted condenser - both necessary features where the haemocytometer is being used - were in part wasted, for difficulty may be experienced in differentiating certain avian blood cells, even when suitably stained and examined under the 1/12" oil immersion with a good source of light.

In one sense, it was remarkable that the principle of haemolysis employed in the standard white cell count for man was not applied to that of birds, for one of the first features observed in the examination of a film made from poultry blood is the large number of degenerate red cells - partially haemolysed. (Indeed, it is intended to present evidence later in this paper to show that such intravascular lysis is a normal feature in the life cycle of the avian erythrocyte). Application of this principle is a simple practical procedure with blood from the domestic hen, for distilled water alone will cause the envelopes of the red cells to rupture, leaving the white blood cells intact.

After this treatment, the counting of the leucocytes in the haemocytometer can be carried out in the usual manner.

This method has been found quite satisfactory, and in the opinion of the writer, is the one to be employed in routine practice when absolute leucocyte counts are required. To facilitate counting, the diluting fluid may be tinted with methyl violet or Jenner-Giemsa.

Where accuracy is not essential, but where a guide as to the approximate number of leucocytes per c.mm. is required, the following method has been found valuable. Take a sample of mammalian blood and determine in the usual manner the number of WBCs per c.mm. From the same source, carefully prepare and stain a film for differential count purposes. Next, observe the number of leucocytes in each microscopic "field," count 10-50 such fields (according to the density of leucocytes) and co-relate the figure obtained with the absolute number per c.mm. determined from the counting chamber.

In the present investigation, the finding of one leucocyte per field represented an approximate figure of 9,000 WBCs per c.mm. If, for example, in the counting of 10 or more fields, there was an average of three white cells per field the sample in question would represent a total count of 27,000 WBC/c.mm. - typical, in fact, of normal hens blood. Owing to the possible unequal distribution of leucocytes throughout the

slide, great care was taken to prepare the film as rapidly and efficiently as possible. A portion of the slide was chosen where the red blood cells were evenly distributed, i.e. not unduly overlapping or widely spaced. This may not always appear possible, and therefore the experimental error can be as high as ± 33%, but application of the method following the making of the normal differential count is preferable, since this gives a general idea as to the numbers of leucocytes present - normal, high or low. After a good deal of practice applying this method to bovine and poultry bloods and checking the results with absolute total counts, the writer's accuracy was found to be ± 15%, which means that it was possible to examine an individual animal's blood daily and have reasonably constant results. It is also a valuable method for recording the general distribution of thrombocytes, and in bloods from diseased birds for calculating the numbers of circulating erythroblasts.

Differential Counts

These have been determined by the counting of 300 or more leucocytes from films prepared on coverslips as well as from ordinary slide "smears," and although the former are to be preferred, slide preparations are better for field work. The slides can be cleaned far quicker than coverslips, and if suitably prepared films are made, the appearance of the cells is very little inferior to that on coverslips. In either event, the first essential is to ensure that the glass is absolutely clean and free

from the slightest trace of grease. When clean, coverslips have been stored in absolute alcohol and dried and polished with a clean soft handkerchief.

The following procedure has given good results for the making of films on glass slides:-

Puncture the vein and using an ordinary sterile or clean bacteriological platinum loop transfer a small drop of blood as quickly as possible to the cleaned slide. Using a clean $\frac{3}{4}$" No: 1 coverslip, place this at an angle of 30° to the slide, the blood drop will quickly flow along the edge of the coverslip and then steadily and evenly, applying light though firm pressure, draw the coverslip towards the other end of the slide. The resulting smear should show the red cells evenly spaced (with little overlapping) and a count of 300 or more leucocytes from such a preparation will be satisfactory for "differential" purposes. Undoubtedly, the features which ensure success are:-

(1) A perfectly clean slide.

(2) Rapid transfer of the blood from the site to the slide, and

(3) Spreading the film by means of a coverslip. This latter allows the operator to control the pressure far more satisfactorily than when using two slides and the resulting film is therefore quite thin and evenly distributed - two essential features for differential and Arneth counts.

Blood Stains

After experimenting with large numbers of stains including Leishman, Romanowsky, Gordon, Gurrs new rapid Giemsa, Jenner-Giemsa, Ehrlichs tri-acid, eosin and methylene blue, the following modification of Pappenheim's panoptic method (after Piney) has been used throughout the work:-

(1) Fix for three minutes in May-Grunvald. Dilute with an equal quantity of distilled water and stain for (a) cattle 1½ minutes, (b) poultry 1 minute.

(2) Wash freely in tap water and counterstain for the following periods (a) cattle 10 minutes, (b) poultry 7 minutes, with:-

To each 2 c.c.'s of distilled water, add 4 drops of Pancrom and 5 drops of methyl-green-orange G.

Finally, wash in tap water, drain or shake off the excess fluid, dry the back of the slide with a clean cloth, and, if required urgently, place in an incubator (37°C.) for 3 minutes, after which the film will be ready for examination.

In the case of coverslip preparations, these are placed film downwards in a watchglass (which avoids stain deposits) and later are transferred direct from the May-Grunvald to the Pancrom stain. If the water is blown vigourously from the completed preparation it dries rapidly in the air and does not require to be placed in an incubator. The mounting of coverslip preparations in green euparol (Gurr) is a distinct advantage over

the usual balsam, especially for the display of leucocyte granules.

Brilliant-cresyl-blue and neutral red have been used for the demonstration of reticulocytes and for vital staining generally.

MICROSCOPY

A Watson "Bactil" microscope with built-in mechanical stage and Holoscopic oil immersion condenser has been used, in conjunction with a Daycol lamp to provide a suitable source of light. It has been found that lamps ordinarily provided for use with microscopes supply too intense a yellow light, therefore the writer has used a series of suitable blue filters which give a good imitation of daylight for constant use. The condenser has been used dry, giving a numerical aperture of 1.0 and an aplanatic cone of 0.92 N.A.

Holoscopic eyepieces giving a flatter and more uniformly defined field than Huyghenian oculars have been used, and in addition to a 2 mm. Holoscopic oil immersion objective (N.A. 1.37) a second has also been available for critical cell examinations.

A Watson and a Swift aluminium screw eyepiece micrometer have been used for cell measurement purposes.

To lessen the eyestrain associated with the making of large numbers of differential and polynuclear counts, the microscope was fitted with a Watson's Universal binocular body, but this was found to decrease the size of the microscopic field, and was therefore discarded.

For the purpose of examining blood cells, the essentials are (a) a constant source of good light, (b) a flat "field" as large as possible, and (c) an easily worked condenser, mechanical stage and fine adjustment.

A GENERAL CONSIDERATION OF NORMAL BLOOD COUNTS

From a survey of the literature, it is evident that some of the standards recommended for animals cannot be considered satisfactory because of the wide variations which exist between the normal counts of different writers dealing with the same species of animal. Presumably, some of the animals believed to be healthy were diseased, or alternatively, the classification of the cells in the differential counts was incorrect.

In any study of normal counts, obviously the first consideration is that of health. In the case of man, it is easy to elicit past history, and a verbal explanation of the present day health of the subject aids the investigation. Latent infections appear to be less common than in animals, and parasites of the intestines and lungs are rare.

Animals are different, for there are usually few records of their past health, and clinical appearances are frequently deceptive. Cattle in excellent physical condition frequently suffer from advanced tuberculosis or from liver fluke, whilst with poultry it is well-known that a number of diseases show few clinical signs recognisable to the layman. Farm stock in general are particularly prone to attack by intestinal parasites, and therefore unless special examinations of the faeces are carried out for the detection of eggs or larvae, it cannot be assumed

they are healthy and free from parasites. The following are some of the diseases which have been found in apparently healthy stock at post-mortem examination:- tuberculosis, Johne's disease, Brucella abortus infection, chronic streptococcal mastitis, liver fluke, parasitic gastritis, bacillary white diarrhoea, coccidiosis, latent fowl paralysis, fatty degeneration of the liver and kidneys.

Therefore, the selection of an animal for inclusion in a standard blood count requires to be made with great care. In the case of poultry, it is easy to carry out a full post-mortem examination at a few minutes notice, so to prove the state of health; then, if abnormalities are found, the blood count can be classed accordingly. This is not possible with bovines, nor is it practical to carry out examinations of faeces for parasitic ova, and therefore one tends to rely on the herd history both before and after the taking of blood counts. If the stock are tuberculin tested, this automatically eliminates one important complication and lessens the work of the investigator.

There is only one other aspect of the subject to be stressed, namely that the rigid selection of animals for complete freedom from parasites is not in itself necessarily wise, for in nature parasitic-free stock are rare. It is well-known that 80% of adult poultry harbour heterakis worms in the blind guts, that coccidiosis can be found in the digestive tract of large numbers of birds of all ages, and that in calves and sheep haemonchus or other trichostrongle parasites of the abomasum or small intestines

are extremely common.

It is, therefore, only necessary to be certain that one is dealing with normal animals, i.e. in good health and free from excess parasites or latent infections.

Throughout the thesis, care has been taken to ensure that animals listed as normal or diseased do definitely belong to the class specifically indicated.

Additional factors to be considered in the making of blood counts refer to (1) age and sex, and (2) the time of day the blood samples were taken relative to feeding.

THE INFLUENCE OF AGE AND SEX ON BLOOD COUNTS

It is well-known that the total and differential blood counts of new-born animals often differ considerably from those of the adult. There are numerous records of the changes occurring in infants, and in bovines Fraser (1930) has studied the subject fully. The writer has confirmed his findings in the calf and also noted those occurring in chicken and pigeons.

Sex, as well as age, also plays a part in causing alterations in the general blood picture. It is generally agreed that the haemoglobin content of the male is higher than that of the female, and also that after castration the erythrocyte count is lowered. Fish and Hayden (1926) showed that in goats the haemoglobin content, total erythrocyte count and percentage of mononuclears fell following castration, whereas the total leucocytes rose, especially the eosinophiles. Sustchowa (1910)

showed a similar fall in the red cell count of castrated one to two year old rams, whilst in chickens Harmon (1936) states that cocks have a higher haemoglobin level than capons, with that of hens lower still. Biely and Palmer (1935) confirm that the mean erythrocyte count of males is significantly higher than that of pullets - a point previously noted by Holmes, Piggott and Campbell (1933) with reference to the haemoglobin content of Rhode Island Red chicks.

DIGESTIVE LEUCOCYTOSIS

Finally, the question of a digestive leucocytosis requires consideration. This was originally considered by haematologists to be definitely related to the time of feeding; later, it was suggested that there was no true leucocytosis of digestive origin, but only a physiological diurnal variation of white cells.

In poultry, Hoppe (1934) has shown that if birds were not fasted a slight leucocytosis occurred about six hours after the consumption of food. If fasted for twenty four hours, a more marked digestive leucocytosis was seen - it occurred in 90% of the birds after taking food, and reached its maximum in approximately 4-5 hours time. The following year, Palmer and Biely (1935) showed that after a 48 hour period of starvation a leucopenia developed, but the same writers also reported (1935a) "While variations have been found in both the erythrocyte and leucocyte count of normal birds, the daily consecutive counts

and hourly counts in individual birds fluctuated around a certain level characteristic of the individual." They specifically state that the normal range of fluctuation in red cells appears to be about 15% per cent.

Blakemore (1934) made blood counts on normal pigeons and fowls at 10.0 a.m. and 3.30 p.m. with interesting results. In both cases, there was a fall in the lymphocytes and concomitant rise in the granulocytes, although this was more marked with pigeons. The absolute leucocytosis shown by the fowls was not outside the range of experimental error, but in pigeons the normal white blood cell (total) count rose from 10,300 to 17,600 per c.mm. in the 5-6 hour interval indicated.

No matter whether the so-called digestive leucocytosis is only a diurnal variation or naturally related to feeding, Harvey and Hamilton (1934) in the Edinburgh Medical Journal state that they consider that even a single differential leucocyte count furnishes a valuable index of bodily condition.

It should be pointed out that in the case of the bovine, since digestion in the ruminant is a continuous process (more so than in ordinary herbivora) due to the rumen and its reserve of food contents, it is understood that no leucocytosis of digestive origin occurs, and if this is so it is doubtful if a true equivalent occurs in poultry, for the crop of a healthy bird is rarely empty during the daytime.

At the 1930 Congress for Microbiology, Kouchakoff brought forward the suggestion that alimentary leucocytosis was really a pathological phenomenon associated with a defense mechanism on the part of the organism against foodstuffs that were unnatural - e.g. milk heated beyond its critical temperature of 88°C., to which was added cane sugar. Unfortunately, there do not seem to be any other papers on the subject confirming this interesting point.

However, in order to avoid any such of the above mentioned disturbances of the leucocytes, the writer has endeavoured whenever possible to take all blood samples from bovines and poultry early in the morning, which is a constant time for practical purposes - i.e. immediately following breakfast.

STATISTICS RELATIVE TO TECHNIQUE

Apart from the above considerations respecting health, age and sex, there is also the important question of establishing a satisfactory method and technique.

Fish (1926) for example believes that there are some advantages in making continued observations on a few animals, compared with the making of single examinations on larger groups. In the opinion of the writer, the first method is wrong if the health of the animals concerned has not been assessed accurately, at some period of the investigation, and, if this is not done,

the second method will fail also, unless the group is sufficiently large to include a preponderance of animals typical of the class believed to be under examination. Probably, the explanation for the common suggestion that large numbers of animals require to be examined before results can be considered satisfactory is due to the fact that no efforts have been made to determine why there have been wide variations from the supposed standards of normal animals. As a fact, "no matter how few animals are examined, or how many examinations are made on each animal, if the technique is good and the animal is known to belong to a definite class, namely healthy or diseased (specifically or generally) then the results are of value." - Extract from Fellowship R.C.V.S. thesis, Blount (1931).

BLOOD SAMPLING

To ensure that the tecnhique of blood sampling was satisfactory, samples from the right and left wings of a bird (P 263) were taken simultaneously.

In addition, from a second bird (R.I.R. 261) counts were made on the first and second drops taken from the same wing vein - the one sample being taken immediately after the other. The differential counts (average of 300 cells), haemoglobin (Tallquist) and estimated total white cell counts (as described earlier in the introduction, p.17) for these two birds were as follows:-

	Case P 263		Case R.I.R. 261	
	Right wing	Left wing	1st.drop	2nd.drop
Haemoglobin	80%	78%	87%	87%
Total W.b.c. count	40,000	36,000	23,000	25,000
Neutrophiles	58.3	57.0	25.0	24.7
Eosinophiles	6.0	5.0	4.0	5.3
Basiphiles	2.7	2.0	2.7	3.3
Large lymphocytes	19.0	15.3	13.3	13.3
Medium lymphocytes	6.7	4.3	8.3	7.7
Small lymphocytes	7.3	15.4	46.3	45.3
Total lymphocytes	33.0	35.0	67.9	66.3
Monocytes	0	1	.4	.4

An examination of the above data shows that for practical purposes these counts are satisfactory, even though they are far from being identical. The greatest discrepancy is between the small lymphocytes in the two samples from bird 263, but it will be seen that the total lymphocyte count is quite satisfactory.

Although it is recognised that the counting of 300 leucocytes is a satisfactory minimum for the making of differential counts in man, owing to possible confusion between certain lymphocytes and thrombocytes it was not clear whether this same figure would be applicable to poultry bloods. In other words,

Differential Count - 2,000 Leucocytes - Reference Bird 2311

TABLE

	P.	E.	B.	L.L.	M.L.	S.L.	T.L.
First 300	40	2.7	2.7	6	10.6	38	54.6
Last 300	36.2	2	4	7	13.3	37.3	57.6
Mean	38.15	2.35	3.35	6.5	11.45	37.65	56.1
First 600	36	3.7	2.0	5.3	12	41	58.3
Last 600	39	1.5	4.0	7.5	13	36.3	57.0
Mean	37.5	2.6	3.0	6.4	12.5	38.65	57.65
700-900	40	2.7	2.7	6	10.6	28.0	54.6
1,000-1,200	33.7	4	1.7	8	11	41.6	60.6
1,300-1,500	40.3	1.3	3.7	10	9.7	35	54.7.
1,600-1,800	36.3	1.7	4.7	8.3	12.7	36.3	57.3
1,900-2,000	36.0	2.0	4.0	5.5	15	37.5	58.0
Second 1,000	36.6	2.25	3.5	7.95	12.1	37.6	57.65
Average 2,000	36.85	2.76	2.9	7.0	11.7	38.7	57.4
+ 10%	40.54	3.04	3.19	7.7	12.9	42.6	63.1
- 10%	33.16	2.48	2.61	6.3	10.5	34.8	51.6
Maximum	45	6	6	11	17	46	63
Minimum	31	0	1	3	8	31	51
Mean	38	3	3.5	7	12.5	38.5	57

is 300 the minimum number of white blood cells required for an ordinary avian differential count, and further, is the distribution of cells by smear preparation sufficiently good for accurate counting purposes?

During the routine examination of a film from a bird suffering from fowl paralysis, 2,000 leucocytes were classified to see whether the distribution of cells over the whole slide was reasonably even.

From the accompanying table it will be seen that the counting of 300 leucocytes is satisfactory for practical purposes because the results are within 10% of the mean. To count 600 cells does not necessarily increase the accuracy of the method, for the average of the last 600 cells enumerated actually shows percentages for the eosinophiles and basiphiles which are outside the 10% limits. On the other hand, to count fewer than 300 cells is unsatisfactory, owing to the wide variations in the distribution of cells.

It would be untrue to suggest that the various leucocytes are evenly distributed - even in a well-prepared film, but if a minimum of 300 cells are counted, uniform results can be expected. The following example shows the differential count obtained from an examination of the edge of a smear contrasted with a second obtained from the central portion of the same slide:-

Light Sussex 2611	Periphery of slide	Centre of slide
Neutrophiles	63.7	63.5
Eosinophiles	.7	.75
Basiphiles	2.0	1.25
Large lymphocytes	5.4	3.5
Medium lymphocytes	11.0	13.0
Small lymphocytes	17.15	18.0
Total lymphocytes	33.55	34.5
Monocytes	0	0

On other slides, three separate counts of 100 leucocytes have shown wide variations, but an average is usually satisfactory. If there has been any doubt, then from 400-600 cells have been counted.

A further example illustrating the degree of uniformity to be expected of the method when two samples of blood are taken from a bird after an interval of several minutes is as follows:-

R.I.R. 2311	First film	Second film
Neutrophiles	35.0	36
Eosinophiles	2.5	3.7
Basiphiles	4.0	2.0
Large lymphocytes	3.0	5.3
Medium lymphocytes	15.0	12.0
Small lymphocytes	40.0	41.0
Total lymphocytes	58.0	58.3
Monocytes	.5	0

In view of the data recorded above on blood sampling, it will be seen that provided a standard technique is employed results which are relatively uniform may be expected.

It is not intended to suggest that blood smear examinations are absolutely to be relied upon, because at times one does find films that appear unsatisfactory, but it is an easy matter to detect these when carrying out the differential count. A second film can then be made and examined in place of the first one, but the necessity for such duplication should be less than 2%.

The great value in carrying out the differential count first is that it gives an immediate insight - during the counting of the first 100 leucocytes - into the general appearance of the film, when important characters in the red or white cells can quickly be noted.

PART II

THE BLOOD PICTURE OF BOVINES IN HEALTH

NORMAL BLOOD CELLS IN THE BOVINE

RED BLOOD CORPUSCLES

Literature

The average size of the red blood corpuscle in bovines is given as approximately 5.3u, and although Fraser, Kohanawa and Berthe carefully indicate that considerably variation in size may occur, others fail to suggest that anisocytosis is a possible feature to be found during an examination of normal cattle blood.

Fraser (1930) states that supravital staining shows the presence of reticulocytes in some calves aged less than two days, but that after that age, none are to be found. Using Giemsa, he demonstrated the phenomenon of punctate basiphilia in calves, but failed to find it in the blood of healthy calves after the age of 48 hours. Stippling was not noted in some calves, even when examined within two hours of birth. Fraser considers the bovine red blood corpuscle to be typically bi-concave, the centre of each corpuscle appearing paler than the periphery in stained specimens. He also points out that although the upper limit for normal red cells is probably 7.8u, a few occur whose diameter is greater - 8.6 - 9.6u; of these, he remarks "These were never found more frequently than one in many fields, and their staining properties resembled those of the other red blood corpuscles. They were not seen in increased quantities in any diseased cattle examined, and as nucleated forms were never observed, classification is difficult.

As they appear to be a physiological occurrence, it seems most probable that they are merely macro-normocytes, and have no relation to the large corpuscles of embryonal blood formation; but no evidence was obtained in support of either possibility."

Concerning normoblasts, whereas Kohanawa (1928) considers none present in adult cattle, Fraser states that they occur at all ages, more frequently in young calves, but even then they are not common.

Original Observations

In the present study of normal cattle, 40 animals have been examined, 14 of which were calves aged from one day to three months.

The average diameter of the red blood corpuscle was found to be 6.5u, which is rather higher than that usually recorded (5.3u) but this may have been due to the fact that coverslip preparations were used, where less shrinkage of cells is believed to occur compared with the using of ordinary smear preparations.

The striking feature noted was the degree of anisocytosis found in healthy cattle - calves and adults alike - so much so to quote an average figure for the measurement of typical corpuscles is rather misleading, for they vary from 4 - 8u. The majority of normocytes range from 5 - 7u, but both microcytes and

macrocytes occur, and their appearance is typical of bovine blood.

Contrary to general experience, the writer has found that large numbers of normocytes display characters which are not indicative of their bi-concave formation. When stained, they show no depression of their centres and therefore the eosin tints they assume are such that each corpuscle appears an even pink throughout. In this connection, it is noteworthy that the largest red blood corpuscles are always fully haemoglobiniferous, whereas the microcytes may be orthochromatic or hyperchromatic.

The quotation above (p. 35) from Fraser shows that he too has noted specially large corpuscles as a physiological feature in bovines, but he prefers to term them macro-normocytes rather than megalocytes, though he admits that no evidence was obtained in support of either possibility.

A study of Plate 17 dealing with Erythroblastosis foetalis in the human (Piney and Wyard, 1938) shows similar features to that in bovine blood (although rather more exaggerated) - i.e. typical bi-concave corpuscles, and others both large and small hyperchromatic in appearance.

There is, therefore, a strong possibility that the cells in question are megalocytes, especially as they are more noticeable in the young than the adult bovine, although they are not oval as described by Davidson and Gullard in the case of megalocytes typical of pernicious anaemia. These writers also point out (p.150) that "the appearance of the red corpuscles in a film

varies somewhat according to the thickness. If the film be thin and evenly spread, the corpuscles generally are uniformly stained throughout. The central concavity or central pallor is not clearly seen as it is in normal blood, and still more so in anaemias of low colour index."

Another interesting feature in normal calves is that the blood platelets are frequently very large. Piney states that giant-platelets are characteristic of Werlhof's disease, whilst Davidson and Gullard note that individual platelets markedly increased in size may be a feature in severe cases of pernicious anaemia.

There is little doubt the whole question of erythropoiesis in the bovine requires careful investigation to decide:-

(1) Whether the large red blood corpuscles are true megalocytes and of embryonal origin.

(2) Why numbers of corpuscles appear with no central depressions, and whether this is due to an artefact in preparation and staining, or associated with their shape, i.e. that they are not really bi-concave. There is perhaps reason to believe that such an investigation would be of value in the general study of haematology relative to pernicious anaemia.

Regenerative Changes

Unlike the blood of most other young mammals and also of chickens, apart from anisocytosis, there are few signs of

regeneration in the new-born calf. There are few polychromasic normocytes, and normoblasts are rare. In an examination of the blood of six calves aged 4-40 hours, during the counting of 1,700 leucocytes, during which about one and a half million red corpuscles were encountered, only one normoblast was noted. These examinations did not disclose any cells with signs of punctate basiphilia, and only the finest degrees of polychromasia were observed in a very small percentage of cells. This is confirmed by the finding of less than 1% reticulocytes in supravitally stained preparations for the youngest calves, and in confirmation of Fraser's work it should be pointed out that some calves even only 16 hours old show no reticulocytes. Only one other normal bovine showed erythroblasts and this, a five day old calf, in which six typically nucleated "cartwheel" normoblasts were noted during the making of a differential leucocyte count. The blood picture in general did not show any other features to indicate that this calf was other than healthy.

Neither poikilocytosis nor meniscocytosis were seen in normal bovine blood, but a few sickle cells were noted in a one day old calf - no such cells have been observed in any other animal - mammal or bird - and they may therefore have been due to artefacts in preparation, although their appearance was typical of the cells seen in Drepano-cythaemia (Piney and Wyard, 1938, Plate XIV).

Adult bovines show few features of note where erythrocytes are concerned for normocytes, punctate basiphilia, polychromasia, (or reticulosis) and poikilocytosis are absent, but the anisocytosis of calves (noted above) is usually to be seen in cattle of all ages.

From these facts it will be appreciated that the normal bovine passes into its blood-stream only mature normocytes, therefore the erythropoietic system shows few features illustrating the adjustment of the young calf to its extra-uterine existence.

BLOOD PLATELETS

A casual examination of the blood platelets in cattle gives an impression that they are mauve-purple in colour, this is actually due to:-

(1) A light blue background - the "cytoplasm," and

(2) A number of fine or large dull azur points - the granules, creating a general hazy mauve effect.

Contrasted with the platelets of man in which the granules are bright azure in colour, these are much more dull and rather dirty in appearance. Also, except in the case of the giant platelets, they are always massed together to agglomerate into a large irregular cell like mass. In the largest platelets, when seen singly, pseudopodia-like projections of the cytoplasm are common.

In some blood films the platelets will be noted to congregate near or around the polymorphs, but not the lymphocytes,

presumably due to some chemio-tactic factor associated with the former cells.

THE NEUTROPHILIC GRANULOCYTE

The bovine polymorph is an interesting cell because of its nucleus, granules and cytoplasm.

The cytoplasm is apparently of an acid nature for it exhibits marked acidophile properties when stained in the usual panoptic manner - as for differential count purposes. As a result, the neutral granules tend to be obscured, and unless very great care is taken, large numbers of films will be prepared in which the neutrophiles are shown apparently free of granules. Even under optimum staining conditions they fail to stain with any intensity comparable with that of man where the polymorph is a cell characterised by its granules. In the bovine, not only are the granules difficult to stain, but they are somewhat fewer in number and smaller in size than in most mammals - appearing as fine neutral or basic points of irregular size.

The average size of a bovine polymorph is about 11u - 12u, which confirms the measurement given by Fraser; but, as with the erythrocytes there is considerable variation in size when large numbers are examined, ranging from about 10.5u - 14.5u.

The nucleus is both polymorphic and polylobular, but the latter feature is not as great as with sheep or in man, where the weighted mean in both cases is seen to be considerably higher

than in cattle. Its many shapes are especially well seen in the various neutrophiles found in the Class I series; the older polymorphs following the recognised procedure of falling into stereotyped classes associated with an increase in nuclear lobes. This morphological complexity of the neutrophile nucleus is discussed under the heading of Modified Arneth Counts.

Using Pappenheim's panoptic staining method, the nucleus is seen to be a bright purple colour with lines of demarcation associated with pale oxy-chromatin. Early forms - metamyelocytes - are less intensely stained, giving a more openwork appearance, whilst the polylobular varieties show a deeper purple - more pyknotic - nucleus with little differentiation of the chromatin. Metamyelocytes are seen more frequently in calves in which up to 8% or 9% may be considered normal, whereas in adult bovines, only 1% or 2% will be found; "Young" forms are rarer still - approximately .5% and .1% respectively. Fraser, Sokolov, Gudin-Lewhowitsch, Sergent and Haffner are all agreed as to the few "Bands" normally found in healthy cattle.

Hypersegmented polymorphs are not common, one five-lobed neutrophile having been noted only in a calf, and during the counting of 3,600 bovine leucocytes for "Arneth" purposes only 31 were found to be in Class IV.

A specific feature of the polymorph nucleus is its rope-like or corded silk appearance following the "band" stage and before true segmentation occurs. It elongates, but still

fails to pass into Class II, because no second lobe to the nucleus has yet been formed. It appears as though the nucleus must continue to increase in length, therefore to remain accommodated within the cell it requires to bend over on itself or to assume other complex twisted positions.

Supravitally stained the neutrophile shows no differential characters additional to those disclosed by the ordinary panoptic method.

Neutrophile myelocytes and earlier forms are never seen in bovine blood in health, and if these cells are noted in differential counts they are always clear evidence of stimulation of the bone marrow.

EOSINOPHILE LEUCOCYTE

The eosinophile is a round cell, larger in size than the neutrophile, being about 12.3u in diameter.

In supravitally stained blood films, the eosinophile granulocytes characteristic appearance is second only in importance to that of the reticulocytes. The relatively large size of the granules and their refractile appearance are outstanding.

Eosinophiles are common in adult cattle, but only about .4% are to be found in calves. Their cytoplasm stains light blue, the granules are numerous frequently overlying the nucleus, although the general conformation of the latter can usually be seen. Indented nuclei of the "band" and early Class I types are common, but "youngs" and myelocyte varieties are also seen in

health in calves, as are those with two lobes to the nucleus. The tightly packed round granules of even size stain a brightish brick red or deep orange colour, but occasionally a few vacuolated areas in the cytoplasm may also be noted.

The nucleus is quite easily distinguished from that of the polymorph assuming royal blue and pale eosin shades with the oxy- and basi-chromatin thus clearly differentiated. It should be noted that the Class I nucleus never elongates like that of the neutrophile but quickly passes to become bi-lobed.

In cows where the percentage of eosinophiles may reach up to 23% in health, "band" forms are again prominent, but segmented types up to Class III may be seen.

In the opinion of the writer, accurate classification of the eosinophiles in terms of the Polylobular or Schilling Counts is impracticable, but Fraser has recorded the following for healthy cattle:-

Class I	"Bands"	Class II	Class III
83.2	43.2	15.7	1.1

THE BASIPHILE GRANULOCYTE

This cell is about the same size as the neutrophile and therefore usually smaller than the eosinophile - i.e. about 11.3u.

The cytoplasm is not usually seen, owing to the space occupied by the nucleus and granules, but occasionally a small portion of it may be noted as a pale blue rim to the cell. The

nucleus also takes on the basic stains, and in contrast to the other granulocytes its colour is blue rather than purple. Its shape frequently appears distorted due to the numerous granules, but it is often monolobular and indented - on one occasion only has the writer seen a basiphile from a healthy bovine with a nucleus comparable with that described by Fraser "the nucleus in most cells (basophiles) appeared to be highly lobulated, the lobes being closely applied to one another, so that the nucleus resembled a bunch of 6-10 grapes."

This writer also described two types of basiphile granules but no such clear cut definition appears possible because they frequently run together and therefore appear to have "fused" like particles of mercury. This feature is even more clearly seen in the basiphiles of the domestic hen. The granules of the bovine basiphile are small, numerous and deep purple in colour; but at the periphery of the cell where the concentration of granules is less than elsewhere, they appear bright azur.

In the examination of 500 leucocytes, whereas two eosinophiles are seen in calves there are usually three basiphiles, but no greater number is found even in adult bovines where the percentage still remains about .6%, to contrast markedly with that of the eosinophiles (up to 25%).

THE LYMPHOCYTES

The lymphocytes of the ox vary greatly in size from about 7.9u - 13.7u, averaging 10.4u, and although there are cells

of all sizes to be found between these upper and lower limits it is convenient to sub-divide the lymphocytes into three groups - large, medium and small varieties. This is purely an arbitrary division, but nevertheless useful from both a clinical and genetic standpoint.

The following characters are common to all lymphocytes:- The cell is round, the cytoplasm is pale blue, devoid of true granules and the purple nucleus is "blobby" in appearance, i.e. the basichromatin appears as "hillocks" among the lighter oxychromatic "dales" which show no organised distribution throughout the nucleus.

Specific variations are as follows:-

The Large Lymphocyte

The average diameter is about 12-14u, its cytoplasm may appear (a) pale blue, (b) polychromasic due to the presence of innumerable fine pale eosin tinted areas intermingling with the ordinary basic cytoplasm, or (c) Türk-type - i.e. deeper, navy blue coloured; occasionally only the periphery of the cell is deeply stained. Such latter "Lympho-Türk" cells (large variety) are common in the adult bovine, but absent in the calf. Vacuolated large lymphocytes are also rare in the calf, but of common occurrence in the cow. This is contrary to the experience of Fraser who has found "nothing in the nature of vacuoles in healthy cattle."

The cytoplasm may contain fine azur granules, but these

are not as common as large lymphocytes with deeply basic cytoplasm nor are they seen in calves.

Owing to the ill-defined character of the nucleus in large numbers of cells there is often very great difficulty in distinguishing some large lymphocytes from small monocytes. The typical "blobby" appearance is much less well defined than in man, many other mammals or chickens. Its shape is typically oval, but kidney-shaped varieties are common, and a few with a fully cleft nucleus are also seen. Such Reider cells have not been noted in the calf.

Large lymphocytes are predominant in bulls, and yearling cattle, and they are also noteworthy in the differential count of calves, but in heifers, milking cows and bullocks the percentage is low.

The Medium Lymphocyte

In the normal maturation of the large lymphocyte the nucleus and cytoplasm both undergo condensation with an associated reduction in size, thus forming one variety of medium sized lymphocyte. On the other hand, if a young large lymphocyte undergoes mitotic division this will also form two medium sized lymphocytes. The Reider cells noted above confirm that nuclear division presumably preparatory to that of the cell is not an uncommon feature in the large lymphocyte.

In the making of a differential count any lymphocyte not typically large or small is obviously one of medium size,

occur in cattle and that they are always found in cells of medium or large size.

In a few cattle, notably calves, the nuclear morphology has been characterised by three or four large plasmosomes and in the same animals these have been present in all varieties of lymphocytes.

Medium sized lymphocytes predominate in calves, heifers and cows.

The Small Lymphocyte

The smallest non-granular leucocyte has a diameter little more than that of the red corpuscle ranging from about 7.9u to 8.9u. The cytoplasm is never more than a narrow rim around the nucleus, though rarely as scanty as in the chicken. If azur masses or granules are present, they often completely hide the cytoplasm. The nucleus is pyknotic and therefore deep purple in colour, and with little or no oxychromatin visible. Small lymphocytes predominate in the bull.

In general, the smaller the lymphocyte the less cytoplasm will it possess, although in comparison with the domestic hen the large lymphocyte of the cow possesses proportionately much more nucleus. A change concomitant with the reduction in size of the cell is seen in the nucleus, the basichromatin of which condenses to the exclusion of the lighter oxychromatin. These are the typical changes of age and confirm the belief that the large lymphocyte is the direct descendant of the lymphoblast and the

precursor of the small lymphocyte.

The percentage distribution of the different classes of lymphocytes in cattle are as follows:-

	% L.L.	% M.L.	% S.L.
Calves	27	42	31
Yearlings	41	42	17
Heifers	16	45	39
Cows	18	55	27
Bullocks	9	44	47
Bulls	81	15	4

The Monocyte

As indicated in the section dealing with large lymphocytes, there is often confusion between these cells and the monocytes, even though the latter are described typically as the largest of the circulating hyaline leucocytes.

The largest mononuclears average about 16.5u, but small ones practically indistinguishable from the lymphocytes are only 13u in diameter, whereas the largest monocytes measure 18u - 19u. The total area of an average monocyte is about six times that of an erythrocyte.

The cytoplasm of this round or oval cell is not typically pale blue as in numbers of the lymphocytes, but it has a pale, slate or polychromasic appearance, due to eosinophilic areas being scattered throughout the pale royal blue cytoplasm. Sometimes

the whole effect is altered owing to the cell having a greater affinity for basic stains than normal, so that a Türk-type of monocyte develops. The deep blue cytoplasm may only be limited to the periphery of the cell.

Azur masses have not been seen in any monocytes from healthy bovines, but fine azur granules are sometimes seen usually close to the main nuclear indentation in monocytes with bean or kidney shaped nuclei.

The nucleus has no constant shape, although the so-called transitional leucocytes of Ehrlich are commonest among the bovine monocytes, but a few have oval or rounded nuclei. An analysis of 100 monocytes showed 60% to be typical transitional leucocytes. In the adult bovine, a few monocytes have been noted with the nucleus having undergone division similar to that in other non-granular Reider cells, but none were noted in calves.

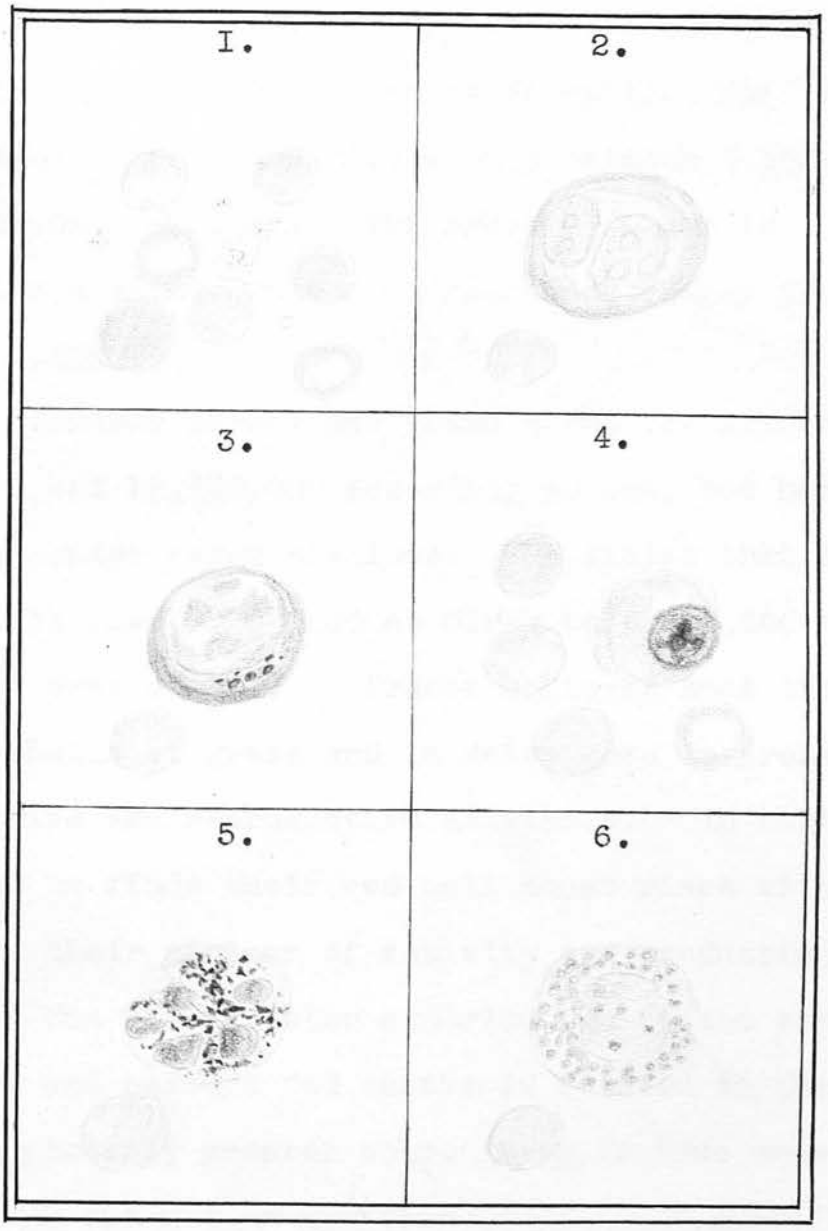
Vacuolated monocytes are rare, a few having been seen in the calf; nor are nucleoli common and these too have only been seen in the calf. No Kurloff bodies occur in bovine monocytes.

The general character of the nucleus contrasts sharply with that of a typical lymphocyte for the basi-chromatin is arranged in fine strands, often at right angles to one another, giving the whole nucleus a linear appearance. Unfortunately, the "hillocky" appearance of the largest lymphocytes becomes indistinct, and therefore considerable confusion may arise in the

differentiation of the two cells. The monocyte is described best as having little nuclear character in comparison with the lymphocyte, and therefore the purple tints it assumes are much more pale, giving it an anaemic appearance.

Monocytes are not common in the bovine, an average of 3% in all types of cattle having been recorded.

Bovine Blood Cells.



1. Erythrocytes and giant platelet.
2. Monocyte with amitotic division of the nucleus.
3. Large lymphocyte.
4. Normoblast.
5. Basiphile granulocyte (Fraser's"bunch of grapes"type nucleus).
6. Eosinophile "band". Arneth type Ta.

THE NUMBERS AND PERCENTAGES OF THE BLOOD CELLS IN HEALTHY BOVINES

Red Blood Corpuscles

The literature on the red cell count in ruminants is fairly uniform with reference to cattle, for ten references on the subject show variations only between 6,183,000 cells/c.mm. and 7,739,000 per c.mm. The average figure is 6,638,100, but for calves a higher number is recorded, namely from 6-10,000,000, average 8,400,000.

Fraser (1930) has found great variations between 4-700,000 and 10,720,000 according to age, but his average is also just under seven millions. He states that in calves the count falls from 7,927,000 at birth to 5,816,000 by the end of the first week of life. Fraser believes that the low counts in yearling bulls at grass and in dairy cows is probably due to their active and reproductive existence. In bulls which are stall fed he finds their red cell count rises with age, associating this with their minimum of activity and production.

One other factor contributing to the variation between stall fed and pasture fed cattle is related to their water intake, which is probably greater where there is free access to it and where stock are not on dry food.

In the present investigation, ten healthy bullocks were examined and their average count was 7,005,200 per c.mm. - a figure quite comparable to that generally recorded. However,

five yearlings housed in an open yard, also healthy, showed marked variations from 5 to 13 millions, averaging 9,685,000 red corpuscles per c.mm. This is therefore in the nature of a plethora comparable with Fraser's stall fed bulls. These results therefore tend to confirm the conclusions stated above relative to diet, exercise and general metabolism.

The Haemoglobin Percentage

Six references in the literature give an average of 63% for cattle and 69% for calves.

Fraser, however, found his average to be higher, i.e. 87% - ranging from 70% to 95%. Even during the first week of life fluctuations between 65% and 85% were recorded. In general, he found the percentage of haemoglobin to fall with age except in the case of bulls, where the average was higher at 83%, his highest figure being 100%.

The writer did not find so low a percentage of haemoglobin in young calves, but in general the findings confirm those recorded. It is not improbable that the higher percentages recorded in this paper and also by Fraser are related to their use of Tallquist's scale which, though less accurate than certain other methods, is of great value in the field. There is often some difficulty when it is first used in assessing the percentage of haemoglobin recorded, but once mastered it proves a rapid method for use on the farm.

In the case of bullocks, the writer found Tallquist's scale inadequate for the majority of those recorded were over 100%, averaging 106%. Similar high figures were found in certain stall fed dairy cows - during the winter months, but in heifers at pasture it only averaged 74%.

A 12% rise in haemoglobin was recorded during the first 14 days in calf 154, i.e. from 72% at birth to 81%. The relationship between newly calved cow and her calf was also interesting, for in one instance mother and calf were alike at 85%, whereas in a second case the percentage of haemoglobin in the calf was from 10%-15% lower than the mother - the latter being just over 100% haemoglobin. This cow however was a very heavy milker, giving 1,900 gallons per annum and therefore her metabolism at calving was specially high.

The Leucocytes

The literature dealing with the total white cell counts of normal cattle shows varieties from 7,475 to 15,750, averaging 8,974 cells per c.mm.

The extremes in Fraser's series of cases are even greater (3,000-15,000) but the average over all his groups is slightly more than 8,000. The highest average 10,200 is for young calves, whilst the lowest 5,200 leucocytes per c.mm. is for yearling bulls. Fraser states "It would appear that the range found in a series of single counts on a group of animals is covered by each animal during the day. There does not appear

to be any indication of diurnal leucocyte tides as reported by Shaw (1927) in man and in the dog; nor is there any indication of a digestive leucocytosis....."

The present series of 30 cases dealing with healthy cattle of all ages confirms the data by Fraser, except in the case of yearlings in which the high average of 15,800 was recorded. Bullocks also showed over 10,000 leucocytes per c.mm., as did young calves. The figure for newly calved cows - 7,470 cells per c.mm. - was surprisingly low in contrast with the 10,600 leucocytes recorded for day-old calves.

The accompanying table contrasts the results of the present investigation with those by Fraser and other workers, the data being classified as accurately as possible according to age groups.

BOVINE BLOOD COUNTS - VARIOUS AUTHORS

	HB %	RBC count	WBC count	
Adult cattle	69	7,739,000	15,750	Maximum
	63	6,638,000	8,974	Average
	56	6,000,000	7,400	Minimum
Calves	74	10,100,000	-	Maximum
	69	8,400,000	-	Average
	64	6,700,000	-	Minimum

BOVINE BLOOD COUNTS - BLOUNT

	HB %	RBC count	WBC count
Calves	82	-	10,060
Yearlings	-	9,685,000	15,800
Heifers	74	-	9,900
Cows	74	-	-
Newly calved cows	87	-	7,470
Bullocks	107	7,005,200	13,716
Bull	82	-	5,600
Average	86	83,450,000	10,425
Animals examined	33	16	38

BOVINE BLOOD COUNTS - FRASER

Calves	76	7,133,000	9,500
Yearlings	64	5,323,000	5,200
Heifers	73	6,610,000	8,900
Cows	65	5,696,000	7,360
Bullocks	-	-	-
Bull	83	8,245,000	6,900
Newly calved cows	-	-	-
Average	87	7,000,000	8,000

Authors cited:-

Creech and Bunyea (1929), Desio (1928), Eichler (1930), Ellenberger and Scheunert (1925), Goodall (1909), Haffner (1926), Huber (1934), Kerekes (1928), Kohanawa (1928), Kuhl (1919), Schwaritz (1920), Simpson (1929), Sokolov (1928-29).

THE DIFFERENTIAL LEUCOCYTE COUNT IN CATTLE

Scattered throughout the literature on veterinary haematology are a number of papers giving the percentage of the different leucocytes in healthy cattle. Unfortunately, few if any give reasons for the belief that the animals are healthy, and as a consequence there are some wide variations. Also age does not appear to have been given much consideration, nor does sex, however the average of eight such references is:-

	Neutrophiles	Eosinophiles	Basiphiles	Lymphocytes	Monocytes
Average	31.5	7.8	.5	53.7	6.6
Maximum	40.5	10.9	1.0	64.0	10.0
Minimum	21.0	5.0	.1	48.0	3.7
Mean	30.75	7.9	.5	56.0	6.8

As in all his investigations Fraser (1930) shows carefully the difference in percentages of cells of each class according to age. Thus:-

The Neutrophiles

Although his average figure is 35%, Fraser states that there are some 64% of polymorphs in the calf during its first day of life. This decreases to 36% by the end of the first week, 31% at four months and 21% at six months. He considers that the high counts of dairy cows (34%) and calves

are probably connected with their high metabolic activity.

In the present investigation, the percentage of neutrophiles in day-old calves was even higher than that recorded by Fraser, for the average was 76.1%, this had fallen slightly to 73.2% by the 41st. hour, and by the end of the first week it was as low as 48%, although the average figure for the first seven days was 65.8%.

Apparently the neutrophile percentage continues to fall for by ten days it was 38.7%, but one calf aged 14 days showed 46% of polymorphs in its differential count. Fraser considers 31% at four months normal, but the writer found only 16% as an average at twelve weeks. This had risen in yearlings, but was still below the 30.3% given by Fraser for animals of this same age. The figure for dairy cattle, 34.7%, coincides with that of Fraser, 34.2%. In bullocks the average was 30.2%, but there were wide variations from 17.3% - 40.3%, and in this instance the higher figure cannot be said to be associated with a high level of metabolism as suggested by Fraser, yet newly calved cows do favour this suggestion for their average for neutrophiles was 39.7%.

The results of the present investigation therefore confirm Fraser's work, indeed if an average of the two sets of figures is taken it gives an interesting insight into the life habits of the polymorph relative to the age of the animals

examined, thus:-

Age	Fraser	Blount	Average
One day	64.1	76.1	70.1
Two days	35.4	73.2	54.3
Seven days	35.9	48.0	41.9
3 months	31.8	16.4	24.1
6 months	21.6	-	21.6
1 year	30.3	23.1	26.7
3½ years	-	30.0	30.0
Bullocks	-	30.2	30.2
Dairy cows	34.2	34.7	34.5
Newly-calved dairy cows	-	39.7	39.7
Bulls	28.2	22.0	25.1
Average	35.0	39.3	37.15

It will be seen that there is a definite alteration in the percentage of neutrophiles according to the age of the cattle concerned. Highest in the day old calf, the adult dairy cow is seen to have considerably less than half this percentage of polymorphs. There is apparently a progressive fall during the first six months of life, after which the neutrophiles rise steadily to reach their second maximum level at calving time.

The high percentage noted in young calves and also in carnivora (throughout their lives) might be associated with the

animal protein nature of their diet, contrasted with the lower number of polymorphs seen in the adult ruminant which is herbivorous. However, there is a marked fall in the percentage seen in older calves long before the diet changes - the protein content of which consists mainly of albuminoids of vegetable origin. Further, by the time the grass diet has become fully established the polymorphs have begun to rise again, so that a direct relationship to the carnivorous nature of the diet cannot be established. However, because colostrum is much more rich in protein than ordinary milk, and since young spring grass eaten by yearling cattle and concentrates fed to dairy cattle are both richer in protein than ordinary grass or hay, there is a possibility that the percentage of neutrophiles is related to the total protein content of the diet. This would also account for the lower percentage of polymorphs seen in bulls which are usually kept short of concentrates when in the byre. Perhaps the specific dynamic factor usually associated with proteins is responsible for the metabolic activity considered by Fraser to be of importance in connection with the subject under discussion.

Compared with the percentage of polymorphs recorded in the literature for bovines generally, i.e. 31.5%, both Fraser's and the present writer's average will be seen to be rather high, but this is no doubt due to the inclusion of data relative to young calves.

For cattle, excluding calves one to two days old, our respective averages are 30.3% and 30.5%, thus showing their similarity to the usually recorded data on this subject.

The Eosinophile Granulocyte

About 8% may be considered normal for the percentage of eosinophiles in cattle according to records in the literature. In this connection, Fraser makes the astounding claim that "The extremes found are from nothing to 25.4% over all groups. This wide range is not due to haphazard occurrence of these cells, but to a definite increase in numbers with age." Yet his own records show only too clearly that milking cows present the wide range of 1.2% to 25.4%, independent of age. He shows that there are few in calves, less than 3% at six months of age, from 1% - 3.4% in yearling bullocks and up to 10% in dry cows. Then he claims "It is clear that there is a graded increase in numbers of eosinophiles from the new-born to the adult animal." The following examples are culled from his own records and disprove these statements quite conclusively:-

Age	Percentage of eosinophiles
One day	.4
One day	1.4
Two days	0
Four days	0
Seven days	0

Age	Percentage of eosinophiles
Nine days	0
2 - 4 months	0
2 - 4 months	3.0
4 months	.8
6 months	0
Yearling bulls	1.0
Yearling bulls	3.4
Cow - aged 3 years	19
Cow - aged 4 years	2.4
Cow - aged 4 years	18.2
Cow - aged 5 years	22.0
Cow - aged 6 years	8.4
Cow - aged 7 years	1.2
Cow - aged 8 years	8.2
Cow - aged 8 years	21.2
Cow - aged 9 years	10.6

There is, therefore, no graded increase relative to age.

The following table shows the percentage distribution of eosinophiles, and their variations for each class of animal examined in the present investigation:-

	%	
Calves - 1 day	.08	(0 - 0.3)
Calves - 7 days	.19	(0 - 0.7)
Calves - 10-12 weeks	.90	(0 - 2.0)
Yearlings	1.90	(0.4 - 4.3)
Heifers	15.40	(6.5 - 23.0)
Dairy cows	17.90	(9.0 - 30.3)
Newly calved cows	8.40	(7.0 - 11.0)
Bullocks	5.70	(1.7 - 10.7)
Bull	6.50	

This data therefore confirms the wide variations shown in the previous table, the figures for which were extracted at random from Fraser's publication.

It also confirms that the percentage of eosinophiles seen during the first year of life is always low - less than 5%, whereas in heifers and dairy cattle the figure may read 25%-30%. Finally, it shows that the eosinophile percentage in bulls or bullocks is nearly always well below that of cows, and it indicates that at calving time there is no eosinophilia.

No data was obtained to show why there were such marked variations in each group, nor yet why there should be any increase at all in the percentage of eosinophiles in any one group. (See discussion on Eosinophilia, p.174).

The results of this investigation did not confirm Fraser's additional suggestion that the percentage of eosinophiles at birth was higher than that of calves a few days old.

The Basiphile Granulocytes

Basiphile leucocytes are never prominent in the blood picture of bovines. The average percentage figure recorded in the literature is .5%, but Fraser only found .2% to be normal. More than half the animals he examined showed no basiphiles, whereas in the present investigation only 30% of the 40 normal animals examined failed to show any basiphiles in their blood. Fraser found none in calves less than 24 hours old, although his highest records were in calves during the first week of life.

The average figure for the present writer was .65%, but the maximum recorded was 2.7% in a 36 hour old heifer-calf - in addition, 2.0% was found in two heifers, and 2.3% in a newly calved cow. Contrary to Fraser's findings, basiphiles were seen in calves only a few hours old. The general distribution was as follows:-

Basiphile Percentage

Calves 1-7 days -	.75	Cows - - - -	.70
Calves 2-12 weeks -	.50	Newly calved cows -	1.10
Yearlings - - -	.60	Bullocks - - -	.33
Heifers - - -	1.25	Bulls - - - -	0

Average - .65%

The Lymphocytes

The lymphocyte is the predominating cell in the differential count of bovines.

In the literature, the average percentage is 53.7%, with a range from 48%-64%. Fraser also finds his average over the whole series of animals to be 53%, but there is a wide range from 13% - 83% recorded, chiefly due to the fact that the young calf is born with neutrophiles predominant. As these decrease with age, so the lymphocytes increase.

The average percentage of lymphocytes in the present investigation was the same - 53.1%, with a range from 21.7% to 69%. The general distribution of lymphocytes for the different groups contrasted with those of Fraser was:-

Age	Fraser	Blount
Day old	29.2	21.7
First week	39.7	42.1
3 months	61.2	67.2
6 months	70.9	-
Yearlings	61.2	69.0
Heifers	-	51.6
Dry cows	66.3	-
Milking cows	44.5	51.7
Normally calved cows	-	46.7
Bullocks	-	60.8
Bulls	53.4	67.0
Average	53%	53.1%

Reference to the literature on the blood of cattle shows that no one has attempted to classify the lymphocytes, yet considerable attention has been paid to the neutrophiles and occasionally also to the eosinophiles in terms of the polynuclear or Schilling counts. Why the granulocytes have received this preference is unknown, except that in man - for whom these counts were originally intended - the neutrophile is predominant in the differential count.

In a study of the blood of cattle and poultry in which the common blood cell is the lymphocyte it is clear that efforts should also be made to determine early forms as well as "shifts to the right." The present method is simple, and, as indicated earlier in the thesis, consists of (1) identifying the large lymphocytes which have just left the bone marrow and which still possess the inherent property of cell division, (2) recognising the progeny of the large lymphocytes as (a) medium sized lymphocytes or (b) small lymphocytes - these latter are the oldest of the circulating lymphocytes, and which through age are pyknotic and shrunken in size.

The distribution of the different classes of lymphocytes in the various groups is as follows:-

Class.	Age	L.L.	M.L.	S.L.	Total
Calves -	One day	11.3	7.8	2.6	21.7
Calves -	One week	12.5	21.7	7.9	42.1
Calves -	Three months	13.0	26.6	27.6	67.2
Yearlings -	One year	28	28.5	12.5	69.0
Heifers -	Three years	8.6	23.2	19.8	51.6
Cows -	4 - 10 years	9.4	28.2	14.2	51.7
Newly calved cows		22.5	15.4	8.8	46.7
Bullocks -	$2\frac{1}{2}$ years	6.3	26.3	28.2	60.8
Bull -	18 months	53.5	10.5	3.0	67.0
	Average	18.4	20.9	13.8	53.1

It will be seen that in calves there is an obvious stimulus to the lymphatic system as contrasted with that of the adult heifer or cow. It can also be noted that at first the blood-stream is not suited for maturation of these early lymphocytes. At three months, the lymphocytes appear to have become more stabilised - a feature also well seen in bullocks and adult cattle.

A further stimulus to the lymphoid system is noted in yearlings and also in newly calved cows, but the excess large lymphocytes noted in the bull may not be typical of the sex. Although quite healthy, tuberculin tested and abortion tested, he had lost one of his horns in a motor accident some four months previously and the lymphocytosis noted may have been related to

this factor.

In an endeavour to express the grouping of the lymphocytes in the form of an index the ratio between large and small lymphocytes may be considered. It is incorrect to leave out the medium sized lymphocytes, because a number of these may be nearly large or nearly small, therefore the following formula is suggested:-

$$LL + \frac{ML}{2} : \frac{ML}{2} + SL$$

Probably it would be more accurate to record five groups of lymphocytes - large, large-medium, medium, medium-small and small, but this does not appear practicable, whereas the three main groups indicated above can be detected easily in stained films.

Using the above formula the ratios for the bovine groups are:-

	LL : SL =	Ratio
Day old calves	15.2 : 6.5 =	2.50 : 1
Week old calves	23.4 : 18.8 =	1.24 : 1
3 month calves	26.3 : 40.9 =	0.64 : 1
Yearlings	42.3 : 26.8 =	1.60 : 1
Heifers	20.2 : 31.4 =	0.64 : 1
Cows	23.5 : 28.3 =	0.83 : 1
Newly calved cows	30.2 : 16.5 =	1.83 : 1
Bullocks	19.5 : 41.4 =	0.47 : 1
Bull	58.8 : 8.3 =	7.08 : 1

The wide ratio seen in calves, yearlings and newly calved cows confirms the stimulation to the lymphatic system as noted above, whilst the narrow ratio in bullocks is due to the predominance of the smaller varieties.

The Monocytes (Large Hyaline Leucocytes)

The rather wide variations in the literature for the percentage of monocytes normally found is probably due to the confusion which exists regarding the differentiation of certain monocytes from large lymphocytes. The range given is from 3.7% to 10%, averaging 6.6%.

Fraser's average for all groups of cattle is 3%, i.e. from 1.4% to 35.2%. He finds the numbers very variable at birth and high during the first week, but this is only transient as from two months of age upwards to the adult bovine the percentage only varies from 2% to 13%. Fraser also finds that fluctuations throughout the day are as wide in hourly counts as in the various groups.

The average throughout the present investigation is similar to that of Fraser - 3.23%, as shown on the accompanying Table.

It will be seen that the highest percentage recorded was in yearlings with the lowest in dairy cows, but there were wide variations within each group.

MONOCYTE PERCENTAGE

	Average %	Range
Calves 1-7 days	2.8	0.3 - 7.7
Calves 2-12 weeks	4.1	1.0 - 8.0
Yearlings	5.3	3.3 - 6.3
Heifers	1.85	1.0 - 2.7
Cows	1.0	0 - 2.0
Newly calved cows	4.1	1.3 - 8.4
Bullocks	2.2	1.0 - 4.5
Bull	4.5	4.5
Average	3.23	

MONOCYTE PERCENTAGE

	Average %	Range
Calves 1-7 days	2.8	0.3 - 7.7
Calves 2-12 weeks	4.1	1.0 - 8.0
Yearlings	5.3	3.3 - 6.3
Heifers	1.85	1.0 - 2.7
Cows	1.0	0 - 2.0
Newly calved cows	4.1	1.3 - 8.4
Bullocks	2.2	1.0 - 4.5
Bull	4.5	4.5
Average	3.23	

THE BLOOD PICTURE OF BOVINES IN DISEASE

THE BLOOD PICTURE OF BOVINES IN DISEASE

BOVINE TUBERCULOSIS

Clinical Notes

Tuberculosis is rife in dairy cattle, mainly associated with insanitary housing conditions, improper ventilation, and the spread of the disease to calves by droplet infection and by tuberculous milk. An additional factor concerns the drain on the animals system through heavy milking and the inadequate feeding of minerals and vitamins throughout the winter months when cattle are housed intensively.

In the calf, lesions of the liver and spleen are present if the tuberculosis is of congenital origin. The commonest form in heifers is a localised lesion of the retro-pharyngeal lymphatic glands causing "snoring" whilst in older cattle pulmonary tuberculosis with involvement of the pleura, lungs, mediastinal and brnchial lymphatic glands is only too well known. As the disease advances it extends to involve the peritoneum, genitals and udder. Tuberculosis is uncommon in the bull, but fully 40% of all untested dairy cattle are reactors to the tuberculin test.

Literature

In spite of the gravity of the problem there are few references in the literature to the blood changes which accompany this disease. Schwanitz (1920) examined seven cases and found four associated with a lymphocytosis, two with a neutrophilia and one with an eosinophilia. From our present state of

knowledge, however, it is evident that the increase of eosinophiles was not primarily of tuberculous origin. Knoblauch (1924) found a marked reduction of the polymorphs from 30% to 2% - 3% in chronic bovine pulmonary tuberculosis. Thijn (1938) states that the significant blood changes are a slight tendency to anaemia and leucopenia. The granulocytes sometimes show a depression of the eosinophiles and neutrophiles associated with many atypical polymorphs and a tendency to monocytosis. He concludes that whilst none of these changes are specifically characteristic, they are thought to serve as a useful indication of the possible existence of tubercle infection.

Fraser (1930) has contributed a most valuable paper on the subject, for he was fortunate enough to be able to study the blood changes after the intravenous injection of living tubercle bacilli into calves causing them to develop miliary tuberculosis. The effect of the inoculation was to cause "a tremendous increase of neutrophile leucocytes within 24 hours from a pre-inoculation level of 1,000 to 4,000 up to 7,000 to 32,000 per c.mm., followed by a fall to normal in 3-6 days and remaining low until death." The lymphocyte reaction in general was more slow, and in six calves that died within 22 days there was an increase of monocytes to at least three times the normal number. Concerning the lymphocyte-monocyte ratio Fraser comments "showing a fall in the ratio after inoculation with tubercle

bacilli, the ratio remaining low or continuing to fall in those calves which died early, rising in those which lived for four weeks and reaching great heights in the two calves which survived." The weighted mean index showed a tendency to shift to the right from a pre-inoculation level of 1.17 to 1.22, whereas the Schilling haemogram showed a marked increase of bands with a few younger forms present. Fraser concludes "that the Arneth index is valueless and frequently misleading as applied to cattle, and that the Schilling haemogram while not so delicate a test nor so easily applied as in the human subject, is of definite value." Fraser also gives as a summary of the conclusions which have been arrived at by independent authorities regarding human tuberculosis that "an increase of lymphocytes and eosinophiles with a decrease of monocytes indicates improvement of the condition, while a decrease of monocytes and eosinophiles with an increase of neutrophiles indicates an extension of the lesions, and the reverse holds true in each case."

However, Basel and Lewek (1928) conclude that an increase of eosinophiles should not be considered a favourable sign nor should a lymphocytosis.

Whitby and Britton (1935) discussing the blood changes in tuberculosis of man point out that in acute miliary tuberculosis most cases have an intensive leucopenia with a moderate degree of hypochromic anaemia, and the sedimentation rate is greatly increased. In acute pulmonary tuberculosis the anaemia develops

rapidly and polymorphs predominate with a total leucocyte count low or slightly raised - again the sedimentation rate is greatly increased. These authors state that the monocyte-lymphocyte ratio is normally 1:3, and that if this decreases in chronic pulmonary tuberculosis it is a bad prognostic sign and vice versa. They point out that this does not apply in the glandular tuberculosis of children.

Piney (1932) considers lymphocytosis good in the early stages, and leucocytosis with neutrophilia a bad prognostic sign.

In the county of East Sussex, some 400-500 cattle are slaughtered annually under the Tuberculosis Order, 1925. The bovine population for the county is 90,000, including some 36,000 dairy cattle. Although there are over 2,200 registered cow-keepers, only 40 hold tuberculin tested licences. The following information abstracted from the Chief Veterinary Officer's annual report (1936/37) is of interest:-

TUBERCULOSIS DATA

	Chronic cough	Udder or milk	Emaciation	Total
1936/37	364	79	49	492
1935/36	273	63	45	381

	Advanced	Not advanced	Not affected	Animals tuberculin tested as an aid to diagnosis
1936/37	219	273	3	13
1935/36	205	178	4	7

ADDITIONAL OBSERVATIONS

Haematology

Six cows in various stages of tuberculosis have been examined, and their blood pictures recorded. Four were clinical cases and two were typical reactors to the tuberculin test, neither of which had reacted to tests previously.

Reactors to the Tuberculin Test

In an examination of eleven non-clinical cases, reactors to the tuberculin test, Fraser (1930) concluded that the shifts in the Arneth and Schilling counts were not sufficiently large to extend beyond the limits for normal animals. One such cow showed a white cell count 200 above normal, the increase being due to lymphocytes.

Following the double intradermal tuberculin injection into an animal free from tuberculosis - i.e. one which will pass the test - the following blood changes may be noted (Table 4, cow 179).

(1) Slight leucocytosis, increase in the percentage of haemoglobin, and shift to the right in the weighted mean index.

(2) Neutrophilia, reduction of eosinophiles and a narrowing of the LL:SL and lymphocyte-monocyte ratios.

Contrasted with this result if the tuberculin is injected into tuberculous cattle the blood changes (see Table 4 heifers 248 and 255) accompanying a positive reaction may be:-

(1) Normal or increased white blood count, with an accompanying normal or decreased percentage of polymorphs, and increase in the haemoglobin percentage.

(2) Reduction in the eosinophiles, and marked widening of the large lymphocyte-small lymphocyte ratio - the percentage of total lymphocytes increases.

(3) The weighted mean index is shifted to the left and there is no increase in bands or young forms of neutrophiles.

It will be appreciated that the eosinopenia, although well marked, is non-specific for tuberculosis as is the increase in the percentage of haemoglobin. The two features which do stand out are (a) the shift to the left in the weighted mean index and (b) the increased percentage of lymphocytes with a notable widening of the large lymphocytes-small lymphocytes ratio due to the influx of large lymphocytes proper. If the above changes can be confirmed in a large number of tuberculin injected cattle - both positive and negative cases - then they may prove of considerable value in assisting the interpretation of skin reactions which are doubtful.

The following represent the main blood cell reactions:-

	Normal heifer	Reacting heifer	Reacting heifer	Normal cow	Normal cow negative to tuberculin test
White blood cells	9,900	13,500	9,000	6,500	8,500
Neutrophiles	30.0	32.0	14.3	27.5	37.3
Eosinophiles	15.4	6.0	6.7	22	8.0
Weighted mean index	1.44	1.25	1.34	1.44	1.57
Large lymphocytes	8.6	39.3	26.3	14	10
Medium lymphocytes	23.2	16.7	37.7	19.5	18
Small lymphocytes	19.8	4.3	10.7	14.5	24
Total lymphocytes	51.6	60.3	74.7	48.0	52
LL:SL ratio	.64 : 1	3.9 : 1	1.5 : 1	.9 : 1	.58 : 1

Non-clinical Tuberculosis

A four year old bullock examined in apparent good health showed at post-mortem examination tuberculosis of the thoracic vertebrae, and lesions of the hepatic and mesenteric lymph glands.

Contrasted with a healthy bullock of approximately the same age the blood picture showed:-

A reduction in the number of red corpuscles but with the haemoglobin percentage unchanged; a leucopenia with increase in the percentage of neutrophiles and eosinophiles and a general decrease in the lymphocytes and a shift to the left in the weighted mean polynuclear index. The monocyte-lymphocyte ratio is

narrower but there is little change in the large lymphocytes-small lymphocytes ratio.

Clinical Tuberculosis

Subject - Cow 211.

A nine year old cow suffering from advanced pulmonary tuberculosis with positive sputum showed the following blood changes:-

Reduction in the erythrocytes - haemoglobin percentage normal, marked absolute neutrophilia and basiphilia, reduction in the eosinophiles and lymphocytes but with the large lymphocyte-small lymphocyte ratio twice as wide as normal due to the percentage of large lymphocytes. Since there is a slight increase in monocytes the monocyte-lymphocyte ratio becomes reduced fourfold from 1:51 to 1:13. The weighted mean index is shifted to the right from 1.39 to 2.10.

Subject - Cow 209.

In this cow also suffering from clinical tuberculosis the most marked features of the blood were:-

Leucopenia with reduction in the percentage of neutrophiles, eosinophiles and lymphocytes. The basiphiles were increased and the monocytes more so with the result the normal monocyte-lymphocyte ratio of 1:51 was reduced to 1:1.9

Subject - Cow 360.

This cow was suffering from advanced generalised tuberculosis - the blood picture was as follows:-

The percentage of neutrophiles was reduced to only 5%, including .5% of bands, the eosinophiles were totally depressed, lymphocytes increased from 51.7% to 94% including both large and small lymphocytes. The monocytes were practically unchanged, therefore the monocyte-lymphocyte ratio had widened from 1:51 to 1:115, whereas the large lymphocyte-small lymphocyte ratio was .38:1 contrasted with .83:1, in the normal dairy cow. Normoblasts were common.

The changes in the differential count noted in these typical cases are shown on the accompanying Table.

Summary of the Blood Changes in Bovine Tuberculosis.

From a survey of the literature, together with the present data on the blood changes in bovine tuberculosis, it is possible to suggest the main features to be expected during the course of the disease.

First Stage

At about the time an animal first reacts to the tuberculin test there will be no constant change in the percentage or absolute numbers of circulating polymorphs, but the weighted mean index will be shifted to the left.

The percentage of lymphocytes will probably be increased, in part due to an influx of large lymphocytes which will be followed by a widening of the large lymphocyte-small lymphocyte ratio - the percentage of small lymphocytes being lower than normal. The monocyte-lymphocyte ratio is not of value at this early stage of the disease.

Second Stage

Following the first reaction to the tuberculin test and with an extension of the disease process, but still in the pre-clinical stage, oligocythaemia develops. The lymphocytosis previously noted gives way to a neutrophilia, although the total leucocyte count may still be little different from normal - the weighted mean index is still shifted to the left. At this stage the monocyte-lymphocyte ratio may be positive (reduced) and therefore of value contrasted with the large lymphocyte-small lymphocyte ratio now normal again.

Third Stage

With the development of clinical signs an absolute neutrophilia is to be expected, and although the total percentage of lymphocytes is reduced further the large variety are increased, therefore the large lymphocyte-small lymphocyte ratio again becomes of value and is seen to be wide. The monocyte-lymphocyte ratio is also positive, but the weighted mean index may be shifted to the right.

Final Stage

In the later stages of the disease, the anaemia is marked by a normoblastic response, and the blood picture is essentially lymphatic with a great reduction in the neutrophiles and complete suppression of the eosinophiles. Neither the large lymphocyte-small lymphocyte nor the monocyte-lymphocyte ratios are positive, due to the retention by the blood-stream of

the small lymphocytes. The polymorphs are so reduced it is impossible to assess a weighted mean index, but since the few neutrophiles noted are Class I type it may be assumed the shift is again to the left.

It is interesting to contrast these changes with those recorded for tuberculosis in the domestic hen - see p. 182.

The above conclusions are not final because of the small number of animals examined, but since each case was typical and confirmed by post-mortem examination it will form an interesting basis for further study.

BLOOD CHANGES IN BOVINE TUBERCULOSIS

	Bullock 202	Normal bullock	Cow 211	Cow 360	Normal cow
Neutrophiles	40.7	30.2	47.0	5.0	34.7
Eosinophiles	11.3	5.7	6.0	0	17.9
Basiphiles	.3	.3	5.0	.3	.7
Large lymphocytes	3.3	6.3	15.0	12	9.4
Medium lymphocytes	19.0	26.3	22.0	27.5	28.2
Small lymphocytes	22.3	28.2	2.0	54.4	14.2
Total lymphocytes	44.7	60.8	39.0	93.9	51.7
Monocytes	3.0	2.2	3.0	.8	1.0
LL:SL ratio	.4:1	.47:1	2:1	.38:1	.83:1
M:L ratio	1:15	1:27.6	1:13	1:115	1:51
Weighted mean index	1.64	1.72	2.10	-	1.39
Degree of tuberculosis	+	-	+++	++++	-
R.b.c's (000's)	5852	7005	4920	-	5696
W.b.c's (000's)	9.5	13.7	17.7	-	7360
Haemoglobin %	105	107	67	-	65

JOHNE'S DISEASE

Clinical Notes

Sometimes termed Paratuberculosis of Bovines, Johne's disease is specific for cattle and sheep and is extremely common in the former animal, notably in heifers. It is characterised by a long incubation period, protracted diarrhoea of sudden onset accompanied by progressive wasting and death. It is caused by an acid-fast bacillus and found to be associated with marked thickening of the ileum and adjacent portions of the intestines, thus preventing absorption of the products of digestion.

Haematology

In the early stages of the disease characterised by slight diarrhoea and sub-lingual oedema the blood picture appears fairly characteristic. The following features were noted in heifer No: 1811 (Table 4).

Leucocytosis with marked increase in the polymorphs including about 5% of bands, and monocytes - 19%. The eosinophiles and lymphocytes were depressed and the weighted mean index shifted to the left. No anaemic features were noted.

At a later stage the neutrophiles and monocytes become depressed, the percentage of bands falls and the eosinophiles and lymphocytes rise. The narrow large lymphocyte-small lymphocyte ratio of .3:1 is characteristic, whilst the weighted mean is shifted well to the left. Anisocytosis is marked, also

crenation - hyperchromatic megalocytes are noted. (See Ref: 1812 and 1813).

When the disease is fully advanced and the animal semi-emaciated the blood picture shows marked erythroblastic changes. Normoblasts are common, also red corpuscles with punctate basiphilia, and there is no reduction in the number of megalocytes noted earlier in the disease. The large lymphocyte-small lymphocyte ratio returns to normal and there is again an increase of monocytes, many of which appear to have engulfed red blood corpuscles. Anisocytosis and crenation are marked. (Reference 1814).

The differential counts for the above cases were as follows:-

	Normal heifer	Ref: 1811	Ref: 1812	Ref: 1813	Ref: 1814
Bands	0.5	5.0	0.3	0.5	2.0
Neutrophiles	29.5	58.5	20.3	24.5	20.0
Eosinophiles	15.4	1.0	8	7.25	3.7
Basiphiles	1.25	1.0	0	.25	0.3
Large lymphocytes	8.6	8.0	7.3	2.5	6.3
Medium lymphocytes	23.2	4.0	19.3	28.5	37.7
Small lymphocytes	19.8	3.5	42.4	36.5	18.0
Total lymphocytes	51.6	15.5	69.0	67.5	62.0
Monocytes	1.85	19.0	2.4	0	10.0
LL:SL ratio	.64 : 1	1 : 1.9	.3 :1.0	.3:1.0	.7:1
Weighted mean index	1.44	1.30	1.04	1.06	1.23

PYOGENIC INFECTIONS

The full details of the blood changes recorded in the following animals are included in Table 5.

Subject 1. Jersey heifer No: 153.

After calving three weeks previous to the examination in question, this tuberculin tested cow suddenly developed a massive oedema of the udder with a temperature of 105°F. A slight teat injury was evident and was considered a likely site for infection.

The writer was called into consultation by the practitioner and a blood examination revealed:-

	Cow No: 153		Normal cow	
White blood cells	6,800		9,900	
Neutrophiles young	5		0	
" band	23	64%	.5	30.0%
" adult	36		29.5	
Eosinophiles	1		15.4	
Basiphiles	0		1.25	
Lymphocytes large	9		8.6	
" medium	14	27	23.2	51.6
" small	4		19.8	
Monocytes	8		1.85	
LL:SL ratio	1.45 : 1.0		.64 : 1	
Weighted mean index	1.02		1.44	

A microscopic examination of the animals milk showed few organisms but masses of pus cells.

It was concluded that the case was one of pyogenic infection of the peri-mammary tissues - the acini and therefore the milk remaining free of bacteria. In view of the leucopenia, large increase of early neutrophiles and depression of the eosinophiles and basiphiles the prognosis was considered bad.

Seven days later the animal was slaughtered, and the only post-mortem finding was a large mass of cheesy pus surrounding and involving the udder. The teat lesion had healed.

Subject 2. Dairy cow No: 217.

This cow was suffering from a typical attack of streptococcal mastitis, but the infection was not severe or fatal.

The differential count and that of a healthy cow were as follows:-

	Cow No: 217		Normal dairy cow	
White blood cells	12,000		7,360	
Bands	1.0	65.7	.3	35
Neutrophiles	64.7		34.7	
Eosinophiles	6.3		17.9	
Basiphiles	.7		.7	
Large lymphocytes	16.7	27.3	9.4	51.7
Medium lymphocytes	9.3		28.2	
Small lymphocytes	1.3		14.2	
Monocytes	0		1.0	
LL:SL ratio	3.6 : 1		.83 : 1	
Weighted mean index	1.10		1.39	

Contrasted with the previous case the prognosis was considered good because the leucocytosis did not include early neutrophiles, there was only "partial" depression of the eosinophiles and a wide large lymphocyte-small lymphocyte ratio characterised by a relatively high percentage of large lymphocytes indicating a good reaction on the part of the lymphatic system.

This case responded to treatment but one "quarter" of the udder remained "light" after recovery.

Subject 3. Shorthorn dairy cow - Marigold No:305.

This cow developed lameness in the right hind foot, soon afterwards a necrotic patch of skin developed anterior to the udder. Neither this place nor the foot responded to treatment and the animal gradually lost flesh, became recumbent and was slaughtered. Post-mortem examination revealed multiple haem-angiomata of the liver which no doubt accounted for the delayed healing process noticeable throughout the whole course of the illness. The foot injury showed laceration of one of the two main deep flexor tendons with some slight pus formation and advanced necrosis leading to perforation. The sole of the foot had apparently been penetrated by a foreign body.

The blood picture showed the following percentages of leucocytes:- Bands 1, neutrophiles 46, eosinophiles 1, basiphiles 0, large lymphocytes 19, medium lymphocytes 17, small lymphocytes 15, total lymphocytes 51, and monocytes 1%. The large

lymphocyte-small lymphocyte ratio was 1.2 : 1, and the weighted mean index shifted to the left to 1.18.

This was considered typical of a pyogenic infection but it failed to give any clue as to the liver dysfunction. The high percentage of large lymphocytes was interesting but it did not appear certain whether this was due to the pathogenic organisms attacking the foot or if this was perhaps a response to the liver failure?

CHRONIC CERVICITIS - BOVINE LEUCORRHOEA

Clinical Notes

Leucorrhoea is common in bovines and is frequently noticed as a post-parturient infection; in a number of cases it is probably associated with interference by laymen at the time of calving. Affected animals usually eat and thrive well but suffer from a typical vaginal discharge consisting of mucus and pus; chronic cases are often not amenable to treatment.

Haematology.

Since the associated micro-organisms are non-specific and frequently of mixed types the blood picture varies accordingly.

Subject 4. Roan Shorthorn cow No: 66.

This cow was tuberculin tested, in good condition, but had suffered from a chronic uterine discharge for several weeks. An examination of the pus showed no streptococci or staphylococci present. After seven weeks treatment she still discharged intermittently and was therefore sold to the butcher dry, and in good condition. The accompanying Table contrasts the blood picture before and after treatment - seven weeks later.

At the time of the initial examination, it can be seen that there was a depression of the neutrophiles and a relative lymphocytosis. As the disease became chronic there was a further depression of the polymorphs and an associated shift to the left in the weighted mean index. The percentage of eosinophiles fell,

whereas that for the large lymphocytes rose, thus making the large lymphocyte-small lymphocyte ratio more narrow and approximately normal. No leucocytosis was present at either examination.

Subject 5. Shorthorn No: 109.

In the second Shorthorn cow (No: 109) also suffering from leucorrhoea, examined at the same time as No: 66, the neutrophiles were increased relatively with the eosinophiles somewhat depressed. The large lymphocyte-small lymphocyte ratio was similar to that noted in the first cow, as was the weighted mean index. No leucocytosis was present.

Cow No: 109 quickly responded to treatment.

THE BLOOD PICTURE IN PYOGENIC INFECTIONS

	Normal cow	Cow 66. Before treatment	Cow 66. Seven weeks later	Cow 109
Bands	.3	0	0	0
Polymorphs	34.7	20	6.7	40.3
Eosinophiles	17.9	12	5.3	6
Basiphiles	0.7	0.7	0.4	.7
Large lymphocytes	9.4	10	18.3	9.6
Medium lymphocytes	28.2	18	37.3	14.3
Small lymphocytes	14.2	38	32	28.7
Total lymphocytes	51.7	66	87.6	52.6
Monocytes	1.0	.3	0	.4
White blood cells	7,360	7,000	7,200	8,000
Haemoglobin	74%	82%	78%	93%
LL:SL ratio	.83:1	.4:1	.74:1	.46
Weighted mean	1.39	1.36	1.15	1.35

BOVINE PASTEURELLOSIS

Clinical Notes

Relatively uncommon in this country, epizootic bovine pasteurellosis has recently been encountered in Suffolk and Sussex. Caused by a pasteurella, it is characterised by spasms of coughing frequently mistaken for Husk - parasitic bronchitis. It is highly infectious, attacks animals of all ages, but responds to suitable chemotherapeutic and vaccine treatment.

Haematology

The writer, who was recently consulted by two practitioners with reference to three outbreaks of bovine pasteurellosis, took the opportunity to examine the blood of half a dozen typically affected animals in different stages of the disease.

In the early stages, there is an increase in the percentage of bands and neutrophiles and there may be an accompanying leucocytosis. In the adult cattle examined, the eosinophiles were relatively high, though below the percentage found in some healthy cows, whereas in calves the eosinophile percentage was low, because in stock under one year of age the number of circulating eosinophiles is always less than 2%. The eosinophilia noted in animals Nos: 270, 271 and 272 therefore is normal and not related to this disease, nor should it be mistaken for a response to parasitic (verminous) bronchitis, so

leading to an incorrect diagnosis.

The lymphocytes though depressed showed a large lymphocyte-small lymphocyte ratio narrower than normal, due to an increase in large lymphocytes. The weighted mean index was shifted well over to the left, for there were few polymorphs present with segmented nuclei, but the percentage of metamyelocytes was less than 10% of the total differential count.

As the disease becomes chronic and the acute symptoms subside the animal tends to improve its general condition and there is a marked reduction in the polymorphs, so that a sub-normal percentage is noted with no increase in band forms.

The percentage of eosinophiles rises - even in calves - and there is a shift to the right in the weighted mean index. The leucocytosis noted in the earlier stages of the disease recedes and leucopenia may follow.

The lymphocytes show a relative increase, but the large lymphocytes-small lymphocytes ratio falls back to normal again, the blood-stream retaining a greater percentage of medium and small varieties.

The accompanying Table shows the series of blood changes described for bovine pasteurellosis.

BOVINE PASTEURELLOSIS

	Ref: 203	Ref: 271	Ref: 272	Ref: 270	Ref: 205	Ref: 204
White blood cells	12,000	15,800	8,200	5,000	7,500	5,000
Bands	5.7	9.3	3	1	0	0
Neutrophiles	61	31	37	25	20	16.5
Eosinophiles	.3	11	11	13.5	15	7
Basiphiles	.3	.7	1	3.5	0	1
Large lymphocytes	5	19	10	14.5	10	7.5
Medium lymphocytes	16.7	9.3	16	16.5	32	28
Small lymphocytes	8	17.7	20	25	20	40
Total lymphocytes	29.7	46	46	56	62	75.5
Monocytes	3	2	2	1	3	0
LL:SL ratio	.82:1	1.1:1	.64:1	.66:1	.72:1	.4:1
Weighted mean	1.23	1.05	1.09	1.05	1.52	1.52

ACTINOMYCOSIS - ACTINOBACILLOSIS - WOODEN TONGUE

Clinical Notes

In the cow, actinobacillus infections involving the soft structures of the mouth are of fairly common occurrence. Formerly called Actinomycosis, it is now realised that this term has a much more restricted use, for the ray fungus usually attacks the jaw-bone proper. In contrast with this, Lignieres actinobacillus is accepted nowadays as the common cause of "Wooden tongue." The typical lesion is one of induration of the tongue and the associated major symptom is salivation.

The Literature

A number of cases of actinomycosis occur in man annually, and the blood picture is stated to show a hypochromic anaemia and leucocytosis - Buchanan (1909). Cabot (quoted Ewing) reports a case where the WBC's fell to as low a figure as 3700/c.mm., although most writers on the subject consider that an absolute neutrophilia is more typical of the disease. Ewing (1903) examining a case of pulmonary actinomycosis reported 21,500 WBC/c.mm., whilst Bierfruend is quoted by this same author as considering a chlorotic anaemia with haemoglobin figures of 30%-50% normal. In a series of four cases at the Massachusetts Hospital involving the liver and lungs, nine leucocyte counts averaged 22,544 - Cabot, 1904. Recently, Whitby and Britton (1935) summarise the position by stating that the invasive stage

of the disease is characterised by a leucocytosis of the order 20,000 - 30,000 per c.mm., of which some 90% are polymorphonuclears. When the disease is extensive, the leucocytosis may be very marked. In fatal forms of the disease, especially abdominal infections, there is a progressive anaemia which terminally may be very severe.

Curiously enough, there are few records of blood counts of cattle suffering from Wooden tongue, therefore the following case is of interest.

Subject

A 34 month old pedigree Shorthorn heifer, in good condition, bought 14 days previously at a Public Auction for 37 guineas, was noticed to be "snoring" and also dribbling saliva from the mouth. A clinical examination revealed considerable sub-lingual oedema, marked induration of the tongue and an extensive accompanying ulceration of the dorsum. The snoring was due to mechanical pressure on the larynx and the case was typical of acute actinobacillosis - Wooden tongue. This heifer had been bred and was bought at a farm where the stock has been consistently tested regularly for Tuberculosis, Mastitis and Contagious Abortion for a number of years. She was, therefore, sold guaranteed free from tuberculosis and abortion, but of course as a maiden heifer only six months in calf the question of streptococcal infection of the udder did not arise. This, then, was a clear cut case of typical uncomplicated actinobacillus

infection of the tongue and associated parts in an otherwise perfectly healthy animal.

The blood pictures of a normal, in-calf, tuberculin tested heifer of approximately the same age and that of the affected animal read:-

	Normal	Wooden Tongue
Haemoglobin	74	78
White blood cells	9,875	13,800
"Youngs"	0	.7
"Bands"	.42	.3
Polymorphs	29.58	28.3
Eosinophiles	15.4	8.0
Basiphiles	1.25	2.0
Large lymphocytes	8.55	19.7
Medium lymphocytes	23.25	28.0
Small lymphocytes	19.75	7.7
LL:SL ratio	1 : 1.6	1 : 0.64
Total lymphocytes	51.55	55.4
Monocytes	1.85	6.3

The following points are noteworthy:- (a) the leucocytosis, (b) the absence from the differential count of an increase in adult polymorphs or metamyelocytes, (c) the marked stimulation of the large lymphocytes and monocytes, (d) the depression of the eosinophiles with the increased percentage of basiphiles, (e) the narrow LL:SL ratio, and (f) the tendency for a shift to the right in the weighted mean of the polymorphs.

The first point noted in carrying out the differential count was the relative absence of neutrophiles, because from a study of the literature, a high percentage of polymorphs would normally have been expected. That a leucocytosis was present was quickly revealed by the finding of 17 white cells in ten microscopic fields, compared with the ten or eleven leucocytes normally found. An absence of "bands" or earlier forms showed the quiescent state of certain areas of the marrow, but an increase in tri-lobed polymorphs accounted for the shift to the right of the weighted mean, since the percentage of Class II neutrophiles was the same in the affected heifer as in a healthy animal. Thus:-

Neutrophiles	Normal heifer	Actinobacillosis case
Class I	59.5	55
Class II	37	37
Class III	3.5	8
Weighted Mean	1.44	1.53

The greatest change concerned the large lymphocytes since there were three times the normal quantity of these circulating in the blood-stream, i.e. about 2,700 per c.mm. It is possible, of course, that the fact the infection of the tongue was actinobacillosis and not actinomycosis was responsible for the lymphatic (monomuclear) as compared with the expected neutrophilic character of the reaction. Accompanying the lymphocytosis was a triple increase in monocytes, indicating

that the effects of the bacillus were not limited to a stimulation of the lymphatic system alone.

Healthy cattle aged three years have large numbers of eosinophiles (12% - 15%) but few basiphiles (0% - 1.25%) in their peripheral circulation, so that in terms of the 8% and 2% respectively found in this case, it suggests that these two functions of the marrow are interdependent, since there was a definite eosinopenia and an absolute basiphilia.

This response of the granulocytes differs somewhat from that which occurs in pyogenic infections, where there is usually complete depression of both types of cells.

Atypical lymphocytes in the form of Reider cells were seen, but if it is accepted that these are merely large varieties of lymphocytes undergoing nuclear division, this only indicates further the activity of the lymphoid system in actinobacillosis.

A few plasma cells - Türk-type - were also present, two of which were the largest and best seen by the writer in any bovine blood smear.

Only a few of the medium sized lymphocytes showed plasmosomes, but large numbers contained azur granules (or masses) and a few showed degenerative vacuoles.

Although it was not possible to carry out a total red corpuscle count, the smears prepared showed no apparent deficiency of erythrocytes, nor were there signs of anaemia or degeneration, other than anisocytosis, which however is a normal

feature in cattle of this age. The majority of the red corpuscles were well formed with depressed centres, very few were crenated, nor were there many megalocytes. Polychromasia and poikilocytosis were absent, as were nucleated red cells, and since the haemoglobin percentage was not lowered there was no reason to suggest that a hypochromatic anaemia was being featured.

Briefly, therefore, the findings in acute uncomplicated actinobacillosis in the bovine are:-

A leucocytosis of moderate degree involving chiefly the large lymphocytes, basiphiles and monocytes, together with an absolute depression of the eosinophiles - the percentage of polymorphs remaining stationary. Anaemic symptoms are not to be expected at this stage of the disease.

CONTAGIOUS ABORTION

Clinical Notes

Epizootic abortion of cattle has severel interesting aspects, notably its widespread nature throughout the dairy cattle of this country, its relationship to Undulant Fever in man, and certain rather unusual clinical features. The latter due to the fact that whereas the causal organism is resident in the uterus and foetal membranes prior to and at abortion, it soon passes to the udder where it remains several months or years. During this time, such carrier cows do not necessarily abort again, but they are often responsible for infecting other cattle, as well as human beings. The blood picture is therefore of particular interest in both the active abortion and latent carrier stages of the disease.

Fraser (1930) reports examinations of five cattle, the blood samples having been taken within a week of the animals aborting - due to specific Brucella abortus infection. Three of the five cows were examined within two days of calving, but this fact did not appear to have been considered by Fraser in his conclusion "that the only alteration observed in the blood of five cases of contagious abortion was a definite lymphocytosis." In addition, no mention was made concerning tuberculosis, so that it cannot be assumed that these cattle were from tuberculin tested

herds, and they were therefore liable to have been suffering from subclinical manifestations of tubercle infection in addition to Bang's disease in its most active state. It was also rather unfortunate that no attention was paid to the question of the class of lymphocyte present - e.g. large, small or vacuolated.

In the present investigation, no facilities were available for the study of cows having recently aborted, but ten cows, known reactors to the agglutination test, and from a tuberculin tested herd where abortions had been experienced on numerous occasions, have been examined. Thus:-

Ref:	Age	Reaction to agglutination test.	Reaction to tuberculin test.	Breed
176	4	Strongly positive	Negative	Jersey
174	4	"	"	Jersey
182	4	"	"	Jersey
187	5	"	"	Jersey
178	5	"	"	Jersey
177	5	"	"	Shorthorn
175	5	"	"	Jersey
188	5	"	"	Shorthorn X Jersey
180	8	"	"	Jersey
179	8	Doubtful - weak positive	"	Shorthorn

At the time of their blood examination, all these animals appeared normal and in good health, and were being used for the suckling of calves - as foster mothers. A number of them had aborted their last calf, but none had recently calved or were suffering from metritis of brucella origin. The agglutination tests (1:50) were carried out at the same time as the differential counts, and the double intra-dermal tuberculin tests about ten days afterwards.

The following table gives a summary of the data obtained, together with similar information from Fraser's cows:-

	A	B	C	D	E
Haemoglobin	74	62.5	60	74	87
White blood cells	7,490	10,600	7,860	9,875	7,467
Polymorphs	22.9	15	34.3	30.6	39.7
Eosinophiles	20.4	6.5	13.1	12.3	8.4
Basiphiles	1.3	.8	.2	1.0	1.1
Lymphocytes	53.1	72.4	44.5	53.1	46.7
Monocytes	2.3	5.3	7.9	1.7	4.1
Weighted mean	1.39	-	1.30	1.41	1.41

A = average of 10 reacting cows - Blount.

B = average of 5 aborting cows - Fraser.

C = average of certain normal dairy cows - Fraser.

D = average of 7 normal cattle comparable in age to those of A - Blount.

E = average of 3 newly-calved cows - Blount.

It therefore appears that the following conclusions may be drawn concerning the blood picture of cattle suffering from Bang's disease:-

Within a few hours of casting her calf, an affected cow will probably show in her blood picture a definite lymphocytosis, the circulating lymphocytes being more than double those normally present, i.e. 7,644 : 3,486 cells per c.mm. There is however no evidence available to indicate which of the three classes of lymphocytes is increased absolutely. This lymphocytosis is sufficiently strong to displace the relative neutrophilia associated with normal calving. An associated fall in the neutrophiles, in terms of both absolute and relative counts is therefore to be expected and occurs as follows:- 1,590 : 2,963 - 15% : 39.7%. The differential count shows no other features of importance.

The percentage of haemoglobin is considerably lower than expected of newly calved cattle, 62.5% compared with 87% Hb, but this feature of the disease is only evident in the early stages, and therefore, as with the lymphocytosis, is probably present as a direct consequence of the typical pathological changes which have occurred <u>in utero.</u> Fraser confirms that the total counts for red cells are normal, i.e. 5,413,000/c.mm., therefore the picture is hypochromic rather than one of oligocythaemia.

Some weeks after calving, the blood picture will be found to have changed, for both the polymorphs and eosinophiles rise, whereas the lymphocytes and monocytes fall to reach their normal level. Although the total leucocyte count is now practically normal, there is an eosinophilia of noteworthy interest - the polymorph "weighted mean" is unchanged. The mononuclear picture in this latent stage of bovine abortus infection suggests that the stimulus which premature calving gives to the lymphocytes is still active, for both the large and medium sized varieties are slightly above normal percentages, with an expected fall in the smallest variety.

Briefly, therefore, the blood changes in contagious abortion of cattle are:-

At calving - An absolute lymphocytosis together with an accompanying oligochromaemia.

Later - Haemoglobin and total white blood cells normal, slight relative lymphocytosis, marked eosinophilia.

These findings are interesting when compared with those of Undulant Fever, where there is frequently a relative lymphocytosis with or without an absolute leucocytosis. (Dalrymple-Champneys, 1929). In Malta Fever also there is a relative increase in lymphocytes, sometimes with a normal leucocyte count (Bassett Smith, 1902). At the height of the fever, Charles (1898) asserts that there is a complete

disappearance of the neutrophiles, only lymphocytes being found in the blood. Whitby and Britton (1935) also confirm that the leucocyte count is typhoidal in type in both Undulant and Malta Fever, and that infection with organisms of the Brucella group produces a moderate hypochromic anaemia - a fact generally agreed by all writers.

THE BLOOD PICTURE OF CATTLE AS AFFECTED BY SULPHANILAMIDE TREATMENT

The importance of the contribution made by "Prontosil" in the treatment of a number of bacterial diseases in man is widely recognised. Its outstanding success in puerperal sepsis and other conditions associated with haemolytic streptococci attracted veterinarians towards its possible value in the control of bovine mastitis, leucorrhoea, and pyo-metritis. A further stimulus was given to the subject when Continental workers reported in 1937 that Prontosil products were apparently valuable in the treatment of Undulant Fever. Similar successes were reported the following year in the British Medical Journal by Lloyd (1938), Matthews (1938), and Page (1938). It is generally accepted that 30% of the dairy cattle population of this country is infected with Brucella abortus organisms, although only about one-third of these actually excrete bacilli in their milk. This widespread nature of Bang's disease (Contagious Abortion) in cattle undoubtedly accounts for the majority of the 400 or so cases of Undulant Fever which are believed to occur annually in Great Britain.

Unfortunately, although the tolerance of mice, rats, rabbits, cats and dogs to Prontosil was determined (in the initial German experiments concerned with its toxicity in 1935) no information as to dosage and tolerance in bovines was available,

nor could any be supplied by the manufacturers.

The writer, therefore, decided to carry out one or two preliminary experiments on cows; and, in view of the reports concerning the production of agranulocytosis and anaemia (as rare sequelae to treatment in man) to check any effects the drug might have on the blood picture of these cattle.

Apart from the information given by Bayer Products in their brochure entitled "Prontosil - a Survey of the New Chemotherapy," May, 1938, suggesting that 1-3 tablets three times daily was reasonable for man, the only other criterion was the fact that it is generally accepted for materia medica purposes that a cow can tolerate sixteen times the dose for dogs - the latter in many respects being considered the same as for man. Therefore, it was calculated that a safe dose for an adult cow would be from 48-144 tablets per day, i.e. 24g. - 72g.

Experiment I

A newly-calved tuberculin tested Shorthorn dairy cow aged seven years (weighing nine cwts.) was given 50 Prontosil rubrum tablets night and morning on five consecutive occasions. No ill effects were noticed and the calf did not apparently take objection to the cow's milk during such treatment. Thus 125 grammes of the azo-compound by mouth appeared perfectly harmless.

Experiment II

"Pulsant," the Jersey cow used in the second experiment, had passed the tuberculin test for several years, but unfortunately

failed in July, 1938. Her skin measurements were:-

25/7/38	Initial measurement	7.0 mm.	
27/7/38	48th hour "	8.3 mm.	
28/7/38	72nd hour "	15.0 mm.	Marked oedema, some pain.
29/7/38	96th hour "	13.5 mm.	Oedematous, some pain.

In view of the previous history, it was felt that the tuberculous infection was probably only of recent origin and not yet beyond the lymph-gland stage. Prontosil rubrum was therefore given in 50 gramme doses, night and morning. After 1,000 tablets (0.5 grammes) had been given, the treatment had to be temporarily suspended, and therefore two days later a second intra-dermal test was carried out, the result being a doubtful reaction, and not a clear cut positive, as previously. Thus:-

6/9/38	Initial measurement	7.6 mm.	
8/9/38	48th hour "	8.5 mm.	
9/9/38	72nd hour "	13.6 mm.	Circumscribed, indurated, painful, no oedema.
10/9/38	96th hour "	12.0 mm.	Nodular, less painful, no oedema.
11/9/38	120th hour "	9.0 mm.	Painless.

It was not expected that the Prontosil would have influenced the tubercle bacilli in such a short time, yet the character of the intra-dermal tuberculin test had undoubtedly changed for the better.

It is hoped to repeat the treatment outlined on "Pulsant" and to observe any effects it may have on the sensitivity of the skin relative to tuberculin.

The following are the blood changes recorded:-

"PULSANT" - Prontosil Treatment

TABLE

	1/9/38	2/9/38	8/9/38	9/9/38
Adult polymorphs	23	29	32	21.3
Eosinophiles	11.7	9	4	6
Basiphiles	.3	1.3	.7	1
Large lymphocytes	17	23	24	27.7
Medium lymphocytes	21.7	18.7	19.7	37.3
Small lymphocytes	24	15	13.6	3.7
Total lymphocytes	62.7	58.7	57.3	68.7
Monocytes	2.3	2.0	6.0	3.0
Haemoglobin	80%	85%	88%	82%
White blood cells	9,000	8,500	5,600	9,000
Reference	287	292	301	303
Remarks	Before treatment.	After second dose.	After 500 grammes Prontosil.	72nd.hour of tuberculin test.

Experiment III

The subject in this instance was a second calf Jersey cow, aged 4½, "Phare Acre II" weighing approximately 8½ cwts. She had repeatedly passed the tuberculin test, but failed the

Brucella abortus agglutination test, and indeed had aborted her last calf, and was therefore considered to represent a typical case of contagious abortion.

As treatment, she was given Sulphanilamide (Glaxo) tablets (0.5 grammes) as follows:-

11/8/38	32 tablets	Blood sample taken before treatment commenced.
12/8/38	96 "	Blood sample taken.
13/8/38	96 "	Blood sample taken.
14/8/38	128 "	
15/8/38	128 "	
16/8/38	128 "	
17/8/38	238 "	
18/8/38	- "	Blood sample taken.
19/8/38	300 "	
20/8/38	- "	Blood sample taken.
23/8/38	Tuberculin test commenced.	
26/8/38	Tuberculin test completed.	Blood sample taken.

During the eight day period of treatment, she received 1,274 tablets, comprising some 637 grammes of powdered sulphanilamide. No toxic symptoms were noticed until about twenty hours after the last dose of 150 grammes, when she was noticed to stumble and show signs of ataxia. In swinging her

head round (attempting to remove a fly from her back) she fell over, and when walking occasionally lost temporary control of her hindquarters. These obvious effects of the heavy dosing lasted about twenty four hours, but it was several days before she could be classed as definitely normal. Presumably, this train of symptoms corresponds to those obtained by Hawking (1937) using sulphanilamide on rabbits, and Marshall et alia (1937) on the dog, since muscular weakness and ataxia were part of the syndrome exhibited by both these species of animals.

"PHARE ACRE" - Sulphanilamide Treatment

TABLE XXI

Day of month (August)	11th.	12th.	13th.	18th.	20th.	26th.
Adult polymorphs	25.3	26	50.0	50.7	54.3	22.3
Eosinophiles	19	24	16.7	18	6.3	7
Basiphiles	1.7	1	1.7	1	0	4.7
Large lymphocytes	10	4	3	8	15.7	34
Medium lymphocytes	28	24	16.7	13	10.7	19.3
Small lymphocytes	11.3	17	9.3	7	5	8.7
Total lymphocytes	49.3	45	29	28.3	31.4	62
Monocytes	4.7	4	2.6	2	8	4
Haemoglobin	73%	79%	80%	89%	98%	84%
White blood cells	9,200	6,800	9,000	8,200	9,000	9,000
Reference	175	189	191	215	222	250

Discussion

In the dairy cow, sulphanilamide compounds seem to effect certain quite definite changes in the blood. Admittedly, it would be unwise to generalise on the findings of these two experiments, but from the data obtained it would appear that the administration of Prontosil and sulphanilamide both cause:-

(a) A rise in the haemoglobin content.

(b) A relative neutrophilia, but not one associated with the introduction of large numbers of metamyelocytes, or any constant shift in the "weighted mean," and

(c) An associated relative lymphopenia.

The neutrophilia resulting from the use of sulphanilamide was more pronounced than that associated with Prontosil rubrum - as was the rise in haemoglobin. (In these experiments, total red counts were not made, but there was no evidence - in the form of polychromasia, poikilocytosis, etc. - to suggest that the marrow had been stimulated to increased erythrocytopoiesis).

These features may have been associated with the particular animal treated, and in this respect, it is interesting to note that the tuberculour cow showed a steady rise in the percentage of large lymphocytes (even though the total lymphocyte count fell), whereas in Phare Acre there was an initial depression of these cells, later followed by a secondary rise at the end of,

and continuing after treatment had ceased.

If it is to be accepted that the large lymphocyte is the primary circulating cell of this series, developing during maturation into the small lymphocyte, then evidence in favour of this can be found from a study of the lymphocyte response of these cows.

In the case of Phare Acre there occurred an immediate depression of large lymphocytes followed by a late, though marked, secondary rise. The medium sized lymphocytes also decreased in number and did not rise again until after that of their precursors, whilst the smallest lymphocytes showed no tendency to rise during the period of the experiment.

Similar favourable evidence was given by Pulsant, where concomitant with the steady rise in the large lymphocytes there was a persistent fall in the smallest variety. The rise in the percentage of "medium" lymphocytes was late, as would be expected if they are to be classed as direct descendants of the large lymphocytes.

In both animals, a marked reduction in the number of eosinophiles occurred, although this was much later in the sulphanilamide case, an additional feature of which was the rise in the basiphiles which occurred after administration of the drug per os had ceased.

The above changes may be summarised thus:-

"PULSANT" (Tuberculosis) Prontosil rubrum treatment	"PHARE ACRE" (Contagious abortion) Sulphanilamide treatment
Polymorphs - Relative neutrophilia	Marked relative neutrophilia.
Eosinophiles - Decrease	Reduction - of late onset.
Basiphiles - No change	Late depression followed by marked rise.
Lymphocytes - General reduction returning to normal. Steady rise of large lymphocytes.	Reduction at first, then relative increase. Steady fall of large lymphocytes, followed by marked rise.
Monocytes - Slight increase.	Decrease followed by later rise.

It will be appreciated that the above short experiments were not carried out with a view to determining whether any veterinary evidence could be obtained to substantiate the claim that the Prontosil group of drugs may be responsible for agranulocytosis - Borst (1937) and Young (1937) - or haemolytic anaemia - Harvey and Janeway (1937) and Kohn (1937). Nevertheless, the limited data obtained showed that neither 500 grammes of Prontosil rubrum nor 637 grammes of Sulphanilamide (administered as a "drench" to cattle over a period of 4-8 days respectively) gave any evidence of causing "a marked fall in the red blood cells and haemoglobin," or of "an almost complete disappearance of polymorphonuclear blood cells." Exactly the reverse in fact, for the percentage of haemoglobin rose, as did the neutrophiles. At the

same time it should be noted that the Prontosil rubrum treatment, in contra-distinction to that of sulphanilamide, did appear to cause a leucopenia, though the white cells quickly returned to normal once treatment ceased.

It was also demonstrated that an apparently satisfactory dose of Prontosil-like compounds for adult cattle is 50 grammes (100 x 0.5 gramme tablets) twice daily, the tolerance limit for sulphanilamide (Glaxo) being reached after the giving of some 1,200 - 1,300 tablets. Kidwani (1938) has shown that mice have a greater tolerability for sulphanilamide than for Prontosil rubrum, so that it does not necessarily follow that cattle can tolerate the azo-compounds as readily as shown above for para-amino-phenyl-sulphonamide.

THE INTERPRETATION OF BLOOD COUNTS IN VETERINARY PRACTICE

The following is the writer's interpretation of the value of blood counts in veterinary practice, notably applied to farm stock and it embodies the results of several years practical experience of the subject in the field and laboratory.

Total Red Corpuscle Counts

As a practical aid to disease diagnosis the making of total red blood counts is seldom essential, but for detecting fine degrees of oligocythaemia, as in early cases of tuberculosis, then total red cell counts are absolutely necessary.

Since there are no primary anaemias affecting the animals concerned, and in view of the fact that secondary anaemias of importance usually show signs of erythropoietic regeneration visible in stained blood films, a critical examination will show cells which are hypochromatic, polychromasic or nucleated, and such films also form a guide as to the numerical relationship between erythrocytes and leucocytes.

Haemoglobin estimations are not of course to be discarded, for they afford definite information as to the oxygen carrying capacity of the blood relative to the available percentage of haemoglobin.

Total White Blood Cell Counts

A useful aid to the estimation of the total leucocyte

count is to calculate the number seen in a given number of microscopic fields.

In the majority of instances an estimated count is just as valuable as an absolute figure, and it also reduces the time factor and technique to be acquired considerably.

Cell Morphology and the Differential Count

What is considered of the highest importance in the examination of blood cells is the necessity for observing definite signs of blood regeneration or degeneration. Immaturity in the red or white cells, early forms from the bone marrow, toxic degenerations and the like must be sought for and recorded. Similarly, a classification of the cells for the differential count is equally important.

Although there is little dispute as to what constitutes erythrocytic regeneration, there are several methods of expressing the equivalent changes in the leucocytes. Chief among these is the Arneth count, and its modification - the Polynuclear count of Cooke and Ponder (1928); and there is also Schilling's (1929) classification of the neutrophiles and eosinophiles to be considered.

The Arneth Count

Unfortunately, a great deal of misunderstanding exists concerning Arneth's (1904) original blood count. This has arisen in part because of the difficulty in translation of

Arneth's voluminous works and also because of its seeming complexity. The following description of it is based on the writer's interpretation of it after personal consultation with Arneth at Münster.

Gruner (1913) in his "Biology of the Blood Cells" shows leucocytes classified according to Arneth, but unfortunately twelve out of the twenty figures depicted are incorrect. Cooke and Ponder (1928) reproduce this plate in "The Polynuclear Count" but do not appear to have realised the mistakes made, nor do they seem to have comprehended Arneth's work any better than Gruner. For examply, they state "After many thousands of observations, we concluded that it was impossible to arrive at constant results by Arneth's method," and "Rayevsky states that 40,000 observations were made before he became accurate, and we have no reason to doubt his statement."

The following is a brief account of the method and principles used by Arneth:-

The classification of nuclei by Arneth's method is utilised for all leucocytes and not merely the neutrophiles, nor yet only for the granulocytes.

No attention is paid to the tinctorial character but only to the shape of the nucleus.

It is assumed that an elongated nucleus or nuclear segment is older than one which is round. This is based on the known morphology of the neutrophile myelocyte nucleus passing

with age through the metamyelocyte stage to the adult polymorph, i.e. from round to indented and elongated, finally to segmented form.

If Ehrlich's tr-acid stain is used, no chromatin filaments will be seen and therefore cells with a segmented nucleus become polynuclear. To each of these "nuclei" Arneth applies the same principle, that of accepting that a "kern" round or oval nucleus will indent unilaterally to become "slingen" or elongated.

If the indentation is slight ("wien") extending not more than one quarter of the way across the "nucleus" this is classed Wa, if almost half way across Wb. Deeper indentations ("tief") are also classed Ta or Tb - the latter being more than three quarters of the way across the nucleus or alternatively a complex deep indentation. (All these indentations are approximate only).

No matter how many nuclei, or nuclear segments, or lobes are seen - (a Panoptic staining method - Jenner-Giemsa - (as later used by Arneth) transforms the polynuclear into a polylobular cell) they are always classed in terms of K or S - round (or oval) or elongated.

All cells with an unsegmented nucleus are therefore of these three main types:-

(1) M = Round (myelocytic)

(2) W = With simple (slight) indentations

(3) T = Deeply indented.

All cells with segmented nuclei are classified into:-

(4) K = With round segments.

(5) S = With elongated segments.

The most hypersegmented nucleus imaginable would be evaluated as "8K 2S" or "5K 5S" or some other combination of K and S, assuming it had 10 lobes to its nucleus.

The accompanying diagrams illustrate typical cells classified according to Arneth.

There are two main criticisms of Arneth's blood count, and both are valid, namely (a) that it is unnecessarily complex, and (b) that the underlying principle concerning the nuclear morphology with reference to age is incorrect.

Arneth's original conception of the changes effected by age has not been proved by experimental work, for none of a satisfactory nature has been carried out.

Ponder (1926) attempted to show the response of the bone marrow to injections of thyroid etc., but as his "normal" rabbits varied quite considerably in their initial counts, and, as the animals were not afterwards subjected to post-mortem examination to prove that they were not diseased, his results are not of great value, and the work should be repeated in another species of animal.

However, even though the fundamental principle underlying Arneth's count is incorrect, he must be congratulated on having stimulated haematologists to study nuclear morphology relative to clinical medicine. A second point of value in the Arneth count is that a special class is reserved for the deeply or complexedly indented unsegmented neutrophile nucleus in which respect the Polynuclear and Schilling counts both fail.

There can be little doubt that many bovine granulocytes with a segmented nucleus (polylobular type II) are younger than others with an elongated monolobular nucleus (Arneth type Tb). It is also certain that large lymphocytes with bean shaped or Reider type nuclei are younger than many medium or small sized lymphocytes with a round nucleus.

The Polynuclear Count

As a means for simplifying the Arneth count this is admirable, but it fails to consider the immature polymorph which is an essential in clinical work, for recognition of bone marrow stimulation is a vital factor to be considered in any toxaemia.

The Weighted Mean Index is, however, valuable for showing all "Shifts to the Right" and also a large number to the left.

The high percentage of Class I polymorphs in the bovine is associated with a complexity of the deeply indented nucleus preparatory to segmentation. Its complicated shape in association with the disposition of the oxychromatin (which

frequently lies parallel to the long axis of the nucleus) causes the appearance of pseudo-filaments. The polynuclear count is therefore not applied to cattle without difficulty, and one reason why some workers have failed to consider this count applicable to ruminants is because of the deception caused by the appearance of these falsely segmented nuclei.

Perhaps the most important feature of the count, and one which has led to some confusion is the definition of what constitutes a lobe. Cooke writes "If there is any band of nuclear material except a chromatin filament connecting the different parts of a nucleus, the nucleus cannot, for the purpose of the count, be said to be divided."

So important is this perhaps a slight amplification is permissible to emphasise the fact that the band of nuclear material joining two lobes shall be chromatic nucleolemma only.

Following the examination of a large number of polymorphs most workers will appreciate that the polynuclear count has academic faults as well as others referable to its clinical application. Owing to the fact that a nucleus has three dimensions and that the investigator has to limit himself ordinarily to two of these, there are frequent occasions when one feels that a cell could exhibit more lobes or filaments than seen from the usual viewpoint. There is, therefore, a grave tendency to place a cell of one class into the next higher so to alter automatically the weighted mean.

From Cooke's wording of the definition above it will be seen that all filaments should normally be of the same thickness, yet there are frequent occasions when filaments in the same cell are seen to differ in diameter. In the opinion of the writer, this is due to the fact that more than the nucleolemma may be joining the lobes.

If the nucleus is visualised as consisting of a membrane with semi-fluid contents, then until the membrane is considered to have squeezed the nucleoplasm into two adjacent portions of the nucleus the cell must be considered undivided, and there will then be less confusion in the interpretation of the count.

For a satisfactory polynuclear count, at least 100 neutrophiles should be counted with the final expression as a "weighted mean."

In an attempt to standardise the width of a filament the writer approached Messrs. W. Watson & Sons of London to manufacture a series of fine wires similar to the one in a screw eyepiece micrometer. At first, this was considered possible, but later it was discarded owing to the spherical aberrations associated with such a method for measuring the width of the inter-lobular nuclear connections.

In practice, it is easier to concentrate on the number of filaments and then add one to the figure obtained rather than to look for the number of lobes or segments present.

THE POLYNUCLEAR COUNT IN CATTLE

The literature on the polynuclear count in animals is not extensive, the first record relative to cattle is apparently that by Simpson (1929) who showed clearly how it differed from man. The following year Fraser also published a few records for normal cattle and calves, but neither author appeared to make use of the weighted mean index for simplifying the expression of their results, which were:-

Neutrophiles

	Class I	Class II	Class III	Class IV	
Simpson	75	22	3	0	Cows
Fraser	73	24	3	0	Cows
Fraser	85.5	11.4	3	.1	Calves

The weighted mean index for cows will be seen to be 1.28 and 1.30 respectively. These are somewhat lower than the present writer's figure of 1.39 (see Table 2), but the general distribution of the cells is similar, in that cells with undivided nuclei are commonest, and Class IV cells a rarity:-

	Class I	Class II	Class III	Class IV
Cows	66	27.8	6	.2

The polynuclear count is excellent for demonstrating the shift to the right which occurs in calves (during the first few days of their life) yearlings and bullocks, when cells with

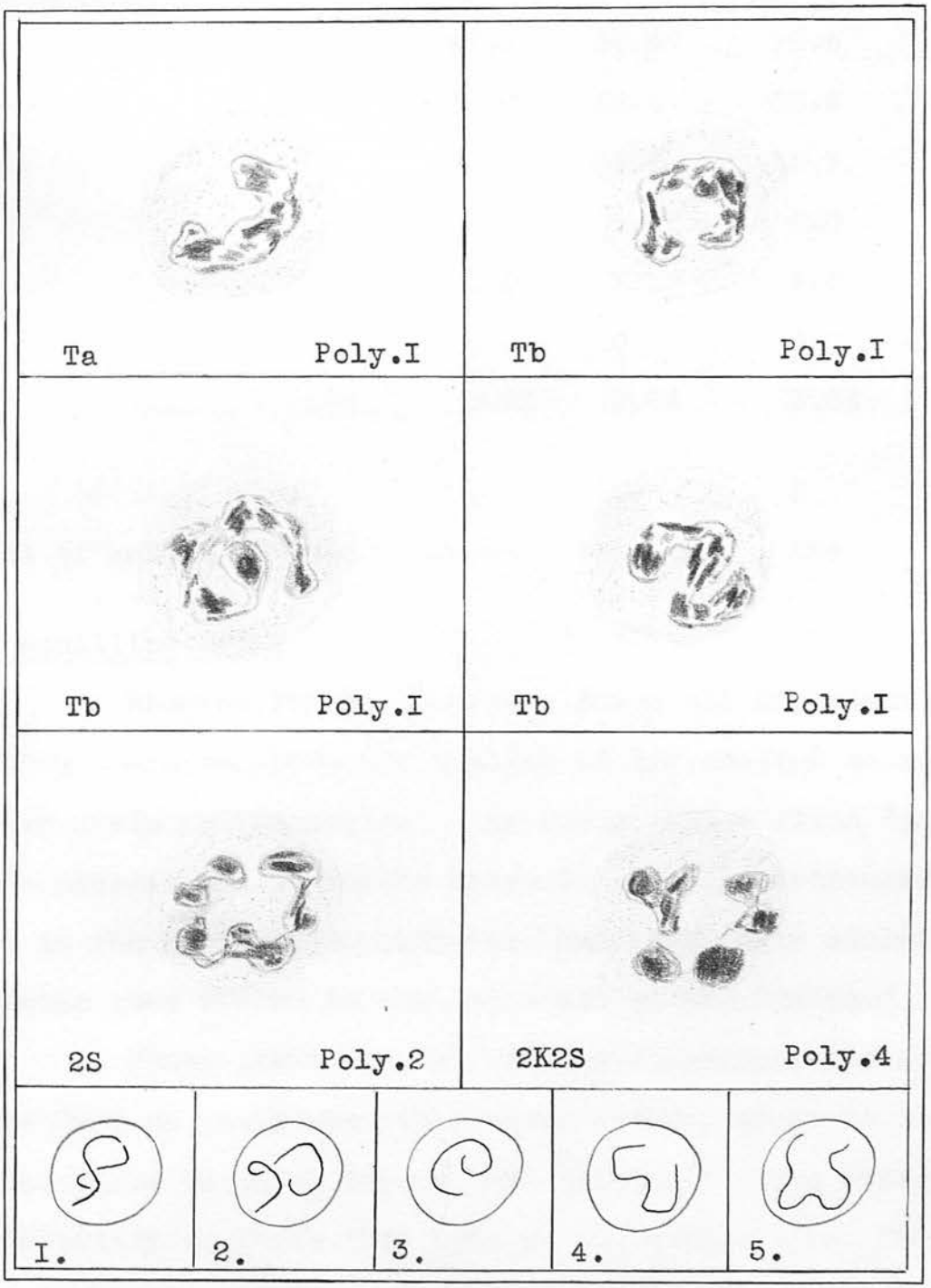
older nuclei are more common than at any other period of life, thus:-

	Class III	Class IV	Weighted Mean
Calves 1-10 days	9.7	1.0	1.63
Calves 2-12 weeks	5.3	0	1.42
Yearlings	12.3	2.1	1.83
Heifers aged 3½ years	3.5	0	1.44
Cows	6	.2	1.39
Bullocks	14.3	1.2	1.72
Bulls	8	0	1.36

THE POLYNUCLEAR COUNT IN SHEEP AND GOATS

Magnus (1926) examined 33 normal sheep and found that the cell distributions were quite different from cattle and resembled those of man. Fraser (1930) confirmed this shift to the right, but also found that it was not as great in the case of day-old lambs - which is contrary to the findings in young calves. The writer has only had the opportunity to examine one healthy ewe, which gave a similar result, whereas a healthy goat showed cells with nuclear segmentation closely allied to that seen in cattle. Thus:-

Arneth and Polynuclear Counts.



Note the elongation of the nucleus of the bovine neutrophile, especially figures 4 and 5 in which the nucleus is still in class I and therefore unsegmented.

	Magnus	Fraser	Fraser	Blount	Blount
Neutrophiles					
Class I	5	25.8	34.4	12.5	73
Class II	32	51.6	35.4	38.5	22
Class III	35	18.4	23.2	36.5	5
Class IV	17	3.2	5.9	8.0	0
Class V	7	1.0	1.1	4.5	0
Class VI	2.5	0	0	0	0
Weighted mean index	2.89	2.22	2.04	2.54	1.32
No: of animals	33	9	10	1	1
Class of animal	Sheep	Sheep	Lambs	Ewe	Goat

The Schilling Count

Whereas Arneth considers shape all important, Schilling is more concerned with the quality of the nucleus as revealed by its staining properties. He visualises a fluid "juice" which flows amongst the chromatin network. It is particularly well seen in the early metamyelocytes, and when their nuclei become pyknotic they shrink to the exclusion of the "juice."

Piney (1928a) p.117, writes concerning the Schilling count "and no toxic change, however severe, makes it impossible to recognise to which type a cell belongs." The writer respectfully suggests that this is not always true, for with a toxic pyknosis the "juice" appears distorted or actually hidden rendering the accurate recognition of "bands" somewhat difficult.

Schilling classified the metamyelocytes into (a) Jugendliche or "young" form comparable with Arneth's Wa type, and (b) Stabkernige or "band" form (synonyms - Stab, Rod or Staff) represented by Arneth Wb and Ta types.

Schilling's evaluation of the earlier neutrophiles would be good were it not for the fact that the differentiation between the deeply indented and complexedly indented nucleus is uncertain and a possible source of error. A survey of the literature shows that many workers fail to distinguish a "band" from the more complex though still unsegmented polymorph. This is Arneth's Class "Tb."

An additional point in Schilling's count is that "although he is probably correct in assuming that he can detect differences in the tinctorial properties of the metamyelocytes associated with age, the recognition of these is most difficult after toxic or other degenerative changes have taken place. At the same time, it must also be remembered that it is impossible to detect accurately any deflection to the right, because Schilling does not differentiate (except mentally) between bi- and polylobular nuclei. The index adopted by Schilling, i.e. the "Kernvershiebung" index is better than the weighted mean because it is the ratio between the myelocytes, "youngs" and "bands" versus the segmented polymorphs. Whereas the weighted mean index of Ponder and Flinn (1926) merely states the average number of lobes per nucleus." - (Blount, 1931).

Fraser has contrasted the suitability of the Arneth index and the Schilling haemogram as applied to cattle, particularly with reference to tuberculosis. (As a fact, he means the polynuclear and not the Arneth count). He concludes the "Arneth index is valueless and frequently misleading as applied to cattle, and the Schilling haemogram while not so delicate a test nor so easily applied as in the human subject, is of definite value as an indicator of myeloid activity."

The writer agrees with Fraser's contention that the Schilling count is valuable for showing early granulocytes, but disagrees with the statement that the polynuclear count is valueless and misleading. Its value can be seen from its use throughout this thesis, but it is of limited use and by no means ideal. As a fact, neither count assesses the polymorph satisfactorily and Arneth's Class "Tb" should be introduced and utilised as a connecting link between the "band" and the Class I type neutrophile. However, if the two counts were so united the weighted mean index could not then be applied satisfactorily. The K.V. index could no doubt be modified to represent the ratio between M, Y and B forms contrasted with the Tb and segmented nuclei types.

Although the original Arneth count was intended for application to all leucocytes, in its modified form the polynuclear count is seldom used except for the neutrophiles.

In the writer's opinion it is equally important to consider the lymphocyte variations and he has therefore suggested a method for computing their clinical value. This is described under the heading referable to bovine lymphocytes on p.46.

Therefore, rather than rely on any one count or index each blood film examined should be recorded on a form or chart which gives the reader a summary of the blood picture at a glance. This should include headings for all main regenerative and degenerative types of blood cells.

PART III

THE BLOOD PICTURE OF THE DOMESTIC HEN IN HEALTH

AVIAN ERYTHROPOIESIS - WITH SPECIAL REFERENCE TO THE DOMESTIC HEN

Unlike the mammalian blood picture in which there is a constant shape and general appearance for the erythrocyte, the normal circulating haemoglobiniferous elements in avian blood are constantly undergoing changes associated with their maturation. Even in the fully haemoglobiniferous erythrocyte the nucleus is not a constant structure, for it either shows changes typical of age, or commences to undergo a degeneration which finally leads to its intra-cellular and intra-vascular disruption. The erythron (Boycott, 1929) is therefore highly complex in birds. A study of the life cycle of the avian erythrocyte may therefore be divided into three important aspects:-

(1) Normal production

(2) Maturation

(3) Degeneration

During recent years the work of Sabin and her colleagues on erythropoiesis has created great interest, but the best insight into red cell production in the domestic hen for clinical purposes is by a study of the marrow and blood cells of the chicks at birth. Direct smears are preferable to marrow sections because the cell characteristics are undistorted and an examination of individual cells is a matter of ease. Since the cells involved in avian erythropoiesis differ considerably from

those of mammals, the following features may be of interest. (Note: In the use of the term "amitotic division" unequal fission of the nucleus is understood to have occurred unless otherwise stated.)

NORMAL ERYTHROPOIESIS

Primary Erythroblast

The stem cell of the erythrocyte series is the only cell which has been observed to show undisputed evidence of karyokinesis in avian blood. It is a large round cell (12u - 15u) with deep basic cytoplasm, but at the time of cell division this may be seen to be faintly polychromasic. Nuclear configuration varies according to the stage of mitosis, but typical figures similar to those represented in Schafer's Essentials of Histology "Karyokinesis of Erythrocyte of Larval Lepidosiren" are common, especially spireme formation and cells showing the formation of daughter nuclei. During the process of the formation of such nuclei their equatorial aspects are distorted by numerous prominent irregular pseudopodia. McGowan therefore was incorrect in assuming that amitotic division was probably the only method of division of the stem cells. Primary erythroblasts are common in the blood of young chick but are never seen in the general circulation of older birds.

Basic Erythroblast

The typical erythroblast of the blood-stream of the healthy young chick (seen also in older birds in cases of severe

anaemia) is also round with a reddish-purple nucleus and with blue cytoplasm. Immediately surrounding the nucleus, a fine, pale blue rim may sometimes be noted. This cell is presumably derived as a direct descendant of the primary erythroblast noted above. It divides by amitotic division so that macro and micro-basic erythroblasts are formed.

Haemoglobinisation

The process by which haemoglobin is incorporated into the erythroblast is characterised by marked changes in the morphology and staining properties of the cell.

The contour of the cell changes to become oval, the nucleus alters similarly and the cytoplasm becomes polychromasic. This cell is considerably larger than the mature erythrocyte and by some would be termed a macro-normoblast. The alteration of cell shape precedes the change in the staining of the cytoplasm from blue to polychromasic. It should be pointed out that throughout erythropoiesis in general there appears to be a distinct co-relation between the shape of the nucleus and the shape of the cell, the former being dominant. In general, the more oval the cell the less round the nucleus, so that by maturity the cell and nuclear conformation are strikingly similar. The polychromasic erythroblast undergoes both regular and irregular amitotic division. Some cells show areas around the nucleus which are unstained. The nucleus itself is purple with pale oxychromatic areas prominent and arranged sometimes in cartwheel fashion.

Under stress of production a small percentage of such polychromasic erythroblasts fail to alter their shape and remain round, therefore the resulting erythrocytes do not become oval as is a feature of normal erythropoiesis.

The series of changes which follow can best be summarised as follows:- Associated with a reduction in cell size is an increased deposition of haemoglobin. The nucleus also condenses to the exclusion of the oxychromatin. Therefore, although a polychromasic erythroblast is larger than a polychromasic erythrocyte the essential difference lies in the character of the nucleus which is more open, less pyknotic and containing more oxychromatin in the former cell. Further, the pale unstained areas close to the nucleus in the erythroblasts usually disappear in the erythrocytes. If these persist, as occurs in a number of secondary anaemias, the cell appears hypochromic.

In a typical polychrome erythrocyte the cell size is still larger than that of the normocyte and the nucleus has a more openwork appearance. When fully haemoglobiniferous the erythrocyte will be seen to have a purple nucleus in which there is little oxychromatin and the basi-chromatin will be found agglomerated, notably at its periphery.

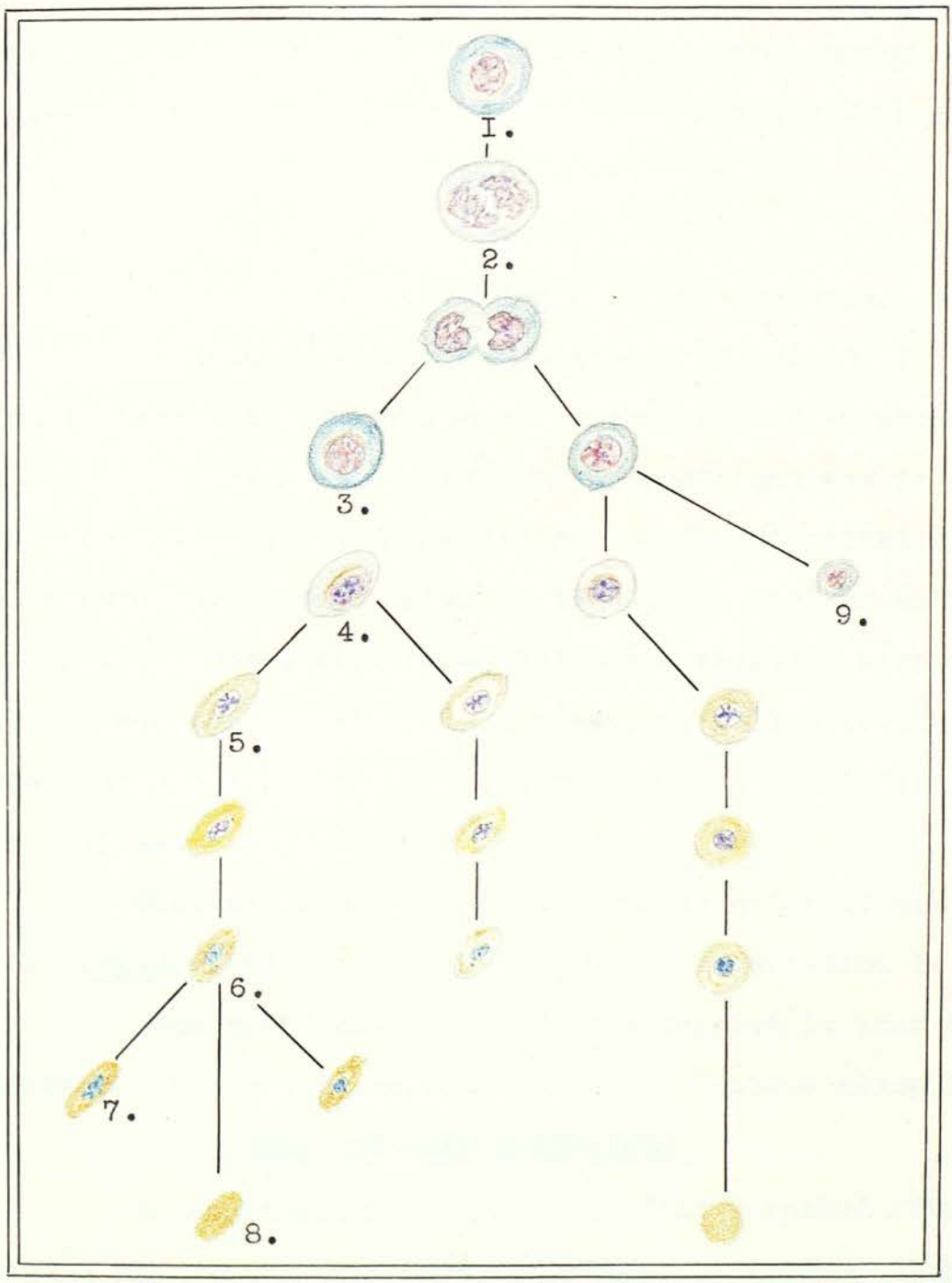
Maturation

The normocyte shows one change in character even when all trace of polychromasia has disappeared in that in the adult

mature cell the nucleus is a dirty blue colour and not purple. On this occasion there is no alteration in the size of the nucleus, nor is there little change in its structural make-up, but in a healthy bird all the mature erythrocytes in the circulation will have deep, rather ink-like coloured nuclei.

A final effect of age is that of pyknosis in which the nucleus condenses so that no oxychromatin at all is available, its oval shape once more tends to become round and to decrease in size. Apparently there is some accompanying ripening of the cytoplasm, because it can be seen to be hyperchromatic and therefore apparently even more haemoglobiniferous than in the normocyte. This is apparently not of megaloblastic origin for the cell is definitely smaller than normal and the densely pyknotic nucleus typically normoblastic. A summary of the changes can be seen in the accompanying diagrams.

Erythropoiesis in the Domestic Hen.



I. Primary erythroblast.
2. Mitotic division.
3. Basic erythroblast.
4. Polychromasic erythroblast.
5. Polychromasic erythrocyte.
6. Normocyte.
7. Pyknosis.
8. Erythroplastid.
9. Micro-erythroblast - Emmel's "haemocytoblast".

The variation in size of normal erythrocytes is not great but in irritative conditions of the bone marrow, notably in haemocytoblastosis and erythroblastosis foetalis anisocytosis is common and even poikilocytosis occurs.

Degenerations

Three main types of degeneration are noted, although two of these should be considered as an end result of regenerative stimuli, namely the formation of notches and of erythroplastids.

The oval contour of the normocyte nucleus is fairly even, even though apparently distorted due to the distribution of the basi-chromatin. In a number of instances, nuclear notches or deep indentations occur, presumably from abortive attempts at amitotic nuclear division. On occasion, full cleavage of the nucleus does occur but it is doubtful whether cell division ever follows.

Nuclear displacement in pyknotic cells is not uncommon, nor is erythroplastid formation (q.v.) - vacuolation is uncommon.

The third main type of degeneration is that of cell disruption, a short description of which follows herewith.

INTRA-VASCULAR HAEMOLYSIS

An examination of any blood film prepared from the domestic hen will show a variable number of nuclear remnants. From one to 140 such remnants occur in every ten microscopic fields - therefore under certain circumstances, intra-vascular

haemolysis is believed to be the main method of erythrocyte destruction, whereas in other cases it only represents one method for normal red cell destruction.

An apparent reversal of the maturation process occurs because the first changes detected are increase of cell size, and enlargement of the nucleus which returns to its purple colour. However, since the haemoglobin is now distributed over a larger area, the general appearance is one of cell anaemia, particularly since the enlarged purple nucleus loses its chromatic character to become ill defined. Further enlargements of the cell take place although the periphery still remains intact, the nucleus continues to increase in size but becomes red purple and devoid of all character. Next, the contour of the erythrocyte becomes lost due to its apparent dissolution in the surrounding plasma until finally only a pale reddish purple nuclear remnant remains to contrast strongly with the normal cells - both red and white - of the blood-stream.

This process suggests a reaction to a hypertonic medium and in some respects resembles the diffusion of the haemoglobin from the red sorpuscles typical of aplastic anaemia (Piney and Wyard, 1928, Plate 15).

A description of this type of erythrocyte degeneration was given by the writer in his thesis for the Fellowship Diploma R.C.V.S.

VITAL STAINING

The erythrocyte in supra-vitally stained films shows important characteristics, notably a reticulum and one or more "Inclusion Spots."

Using brilliant cresyl blue, the cytoplasm of erythrocytes appears pale green yellow, with the nucleus powder blue. The reticulation appears after 10-20 minutes and varies in intensity in different cells, but shows few features different from that seen in mammalian films vitally stained.

Close to the nuclei in typical cells are seen certain inclusion spots, these are specific black dots (usually one per cell) frequently seen showing **Brownian** movement within the cell. Such inclusion spots stain before the reticulum and are demonstrable in large numbers of erythrocytes by the routine pan-optic staining method. Thrombocytes also appear to possess a number of similar inclusions which appear outstandingly basic contrasted with the fine "points" seen in the lymphocytes.

If blood films are stained panoptically before they are dry a peri-nuclear reticulation can be observed, the material is tinged green colour and tends to form a ring around the nucleus. This form of reticulation is identical with that in vitally stained films and is more prominent in immature erythrocytes. Since this reticulation occurs in nucleated erythrocytes and in erythroplastids it confirms the view that it is independent of

the nucleus and not of chromatic origin.

Pigeons blood exhibits similar cell inclusion spots - sometimes double and shadow-like, and also large numbers of reticulated erythrocytes in which the basic reticulum appears more pronounced than in the hen. It is often in "star" formation and of a feathery nature, sometimes occupying the whole of the cytoplasm. Erythrocytes of pigeons are longer and narrower (14.2 x 6.7u) compared with those of the domestic hen (11.8 x 7u), and the majority, if not all, show some degree of reticulation in their cytoplasm.

ERYTHROPLASTID FORMATION

Literature

In view of the widely divergent opinions expressed regarding the fate of the normoblastic nucleus it is of interest to contrast (a) erythroplastid formation and (b) the destruction of erythrocytes in avian and mammalian bloods.

From several points of view the mammalian normoblast can be compared favourably with the avian normocyte because many become fully haemoglobiniferous and both are nucleated cells. Indeed, apart from the question of shape the only main difference is that the avian cell does not normally lose its nucleus and therefore it never becomes a corpuscle.

There appear to be three main theories as to the method by which the mammalian normoblast loses its nucleus (a) by solution, (b) by extrusion, and (c) by karyorrhexis.

Pappenheim (1914) considers that as the nucleus grows older, it becomes pyknotic, owing to shrinkage, thickening of its chromatin and disappearance of its parachromatin. The complete disappearance of the nucleus is believed to be accomplished by a peripheral melting away and by chromatolysis of the adult pyknotic nucleus.

Schilling (1912) published an interesting account in the Folio Haematologica of his researches on the structure of the erythrocyte. As a result he concluded that the erythroblastic nucleus is easily extruded but that this only occurs in

extreme pathological conditions and in the embryo. He is of the opinion that any evidence of intracellular solution of the nucleus should be regarded as due to bad staining, and that the alleged disappearance of the nucleus by karyorrhexis is either a pathological process or quite undemonstrable.

Whitby and Britton (1935) state that the nucleus of the normoblast is extruded from the cell, or in some cases possibly destroyed by solution and fragmentation. They emphasise further the extrusion of the nucleus in the formation of the reticulocyte, and also quote Valentine (1928) who has sometimes seen the free nuclei of normoblasts in the blood-stream. Ehrlich considered this to be the regular way of disposing of the normoblast nucleus, but Cabot (1914) considers this appearance an artefact and believes that absorption or degeneration of the nucleus are normal. Jordan (1924) discussing the subject states that Howell has described its extrusion either as a single or fragmented body in the cat, whilst Emmel (1914) has described for the pig a process by which the red blood corpuscle arises through budding - leaving a nuclear remnant. A diagram illustrates the successive stages in the constriction of the cytoplasm of an erythroblast ending in the formation of a typical corpuscle in the pig. Carleton editing the 1934 edition of Schafer's Essentials of Histology is careful to note that it is uncertain whether the nucleus becomes extruded or simply undergoes absorption. Turnbull (1936) writing on normoblastic

erythropoiesis in Vaughan's publication "The Anaemias" also states that the nucleus is apparently lost by extrusion. An absolutely contrary opinion is expressed by Davidson and Gulland (1930) who write "The ultimate fate of the nucleus still remains unsettled, but it appears to us much more likely that it is dissolved intracellularly than that it is extruded." A special chapter in their book "Pernicious Anaemia" is devoted to the subject of the fate of the erythroblast nucleus. This includes reference to Cooke's work on the ultimate fate of the nucleus, who agrees with their view. He considers pyknosis and karyolysis to be the usual stages preceding the disappearance of the erythroblast nucleus. The following sentence by Cooke is important "The normoblast is sometimes illustrated and described as extruding its nucleus. The molecular disruption preceding this phenomenon is very difficult to imagine and, if it ever does occur, must be the unique example of cellular hara-kari in biology."

ERYTHROPLASTID FORMATION IN BIRDS

Haematology

Normally, of course, all circulating red cells in the domestic hen are nucleated and therefore red blood corpuscles as such are non-existent in poultry. However, careful observation has shown that under certain conditions a small percentage of the erythrocytes do lose their nuclei to form erythroplastids. Conclusive evidence has been obtained proving that the formation

of such corpuscles is by nuclear extrusion and not by intracellular dissolution. Such erythroplastid formation has been observed in young chicks, normal pigeons, "chilled" chicks aged 4-8 days, and in the following diseases - fowl paralysis, infectious catarrh, coccidiosis, intestinal parasitosis, tuberculosis, bacillary white diarrhoea, Blackhead in Turkeys - also in birds following injections of liver extract.

The greatest incidence of erythroplastids in the domestic hen was seen to be in young chicks a few days old. Actually the younger the chick the more red "corpuscles" noted, in fact in chicks still "in-shell" - during the late stages of incubation and at hatching - during the making of a differential count as many as 10-12 erythroplastids may be seen. In diseased birds the greatest numbers have been noted in cases of coccidiosis, fowl paralysis and tuberculosis, but seldom in the numbers noted in healthy day old chicks. In the latter animals the blood usually contains a number of erythroblasts but there is no direct association between the percentage of erythroblasts and erythroplastids, indeed in some diseased birds corpuscles are noted when no nucleated precursors of the erythrocytes are to be found in the blood-stream.

Although the majority of erythroplastids are oval and similar in size and shape to the normal erythrocytes a few are round and presumably have been derived from round erythroblasts of which there are a few demonstrable in the marrow of

most birds. (See erythropoiesis).

An interesting feature in the formation of erythroplastids is the actual extrusion of the nucleus, each stage of the process can be found in stained films as indicated in the accompanying diagrams.

ERYTHROPLASTID FORMATION

Note the nuclear extrusion leading to the formation of red blood corpuscles in the hen, i.e. erythroplastid formation.

In most instances the extrusion of the nucleus may be said to be polar, and although that portion of the cell immediately posterior to the nucleus is seen to be free of haemoglobin, following extrusion an adherent portion of haemoglobiniferous cytoplasm may often be noted. In some instances the nucleus is definitely pyknotic - a point which can be

confirmed either before or after nuclear extrusion - but in other cases it appears little different from those of the neighbouring normocytes. Quite often the resulting erythroplastid is hyperchromatic, only occasionally does it show a depressed centre comparable with the mammalian red blood corpuscle. The "inclusion spot" noted in the chapter on avian erythropoiesis does not necessarily disappear with the nucleus and therefore its structural independence is clear. Poikilocytic avian erythroplastids are seen on rare occasions.

Each microscopic field of normal avian blood contains approximately three leucocytes, and as the ratio between erythrocytes and leucocytes is about 1:100, during the making of a differential count it is possible to examine some 30,000 normocytes. From one to ten plastids may be noted so that the porportion of red blood corpuscles to red blood cells in the domestic hen varies from 1:30,000 to 1:3,000. If it can be accepted that the life of an erythrocyte is not more than 21 days, then it is clear that erythroplastid formation is not a normal method for the self-destruction of the avian normocyte, for the renewal rate in an adult five pound hen is approximately 17,000,000 erythrocytes per day. At the same time this demonstration of erythroplastid formation in birds tends to disprove Cooke's statement that "the molecular disruption preceding this phenomenon is very difficult to imagine, and, if it ever does occur, must be a unique example of cellular

hara-kari."

It is a little difficult to decide whether plastid formation in the domestic hen should be considered a regenerative or degenerative phenomenon. From an examination of erythrocytes undergoing nuclear extrusion the impression is gained that the normocyte nucleus is held in position by a network - possibly that which forms the reticulum of the polychromasic normocyte. If erythropoiesis is hurried then the structural make-up of the erythrocyte may be defective, which would allow of the following explanation of corpuscle formation in birds.

The phenomenon is to be regarded as evidence of regeneration, with each affected cell structurally deficient due to a weak nuclear reticulum. This holds the nucleus in its central position only so long as the cell does not encounter undue pressure on its walls. In the preparation of a blood film this network breaks mechanically, the nucleus slips to one pole and under pressure becomes extruded, frequently carrying with it a small portion of adjacent cytoplasm. That it is not due solely to mechanical disruption is shown by the fact that it does not occur more than 1 cell in 3,000 - a ratio far too wide if the haematologist himself is to be blamed for the "artefact." The high percentage of erythroplastids noted in day-old chicks and in diseases where anaemia is present, contrasted with their absence in healthy adults (hens and pullets), suggests that it is definitely associated with faulty erythropoiesis of regener-

ative origin.

The fact that certain cells undergoing erythroplastid formation have pyknotic nuclei is presumably due to the fact that they have not encountered "bruising" (during their life in the peripheral blood circulation) sufficient to cause dislodgement of the nucleus.

THE LEUCOCYTES OF THE DOMESTIC HEN

The Neutrophiles

These are characteristic of the species because of the shape and colour of the granules. In comparison with those of mammalian leucocytes they may be considered to represent organised components of the cytoplasm. They are long boat-shaped granules in the form of bacilli or rods, hence the term "pseudo-eosinophilic-rods" used by some authors - in contrast with "eosinophilic granules" of the eosinophilic granulocytes.

The average size of a normal neutrophile is 10u, but in haemocytoblastosis, fowl paralysis, etc. following amitotic cell division small varieties are seen about 8.5u - 9u in diameter. Normally, the granules fill the cell, overlie the nucleus and so hide its morphology - thus rendering a polynuclear count difficult.

In older birds, notably hens suffering from advanced fatty degeneration of the abdominal organs, numbers of neutrophiles show refractile "granules" scattered amongst the rods, and where the cells have ruptured these are seen to represent spore-like lipoidal vacuoles in the granules - frequently sub-terminal in position.

The Eosinophiles

Approximately of similar size (10.8u) to the neutrophile, the true eosinophile is sometimes difficult to distinguish.

Two important features are (1) the granules are round, slightly refractile and of even size, (2) their staining is paler, clearer and more yellow-golden or straw coloured. The neutrophile rods are dirty golden-brown tinted.

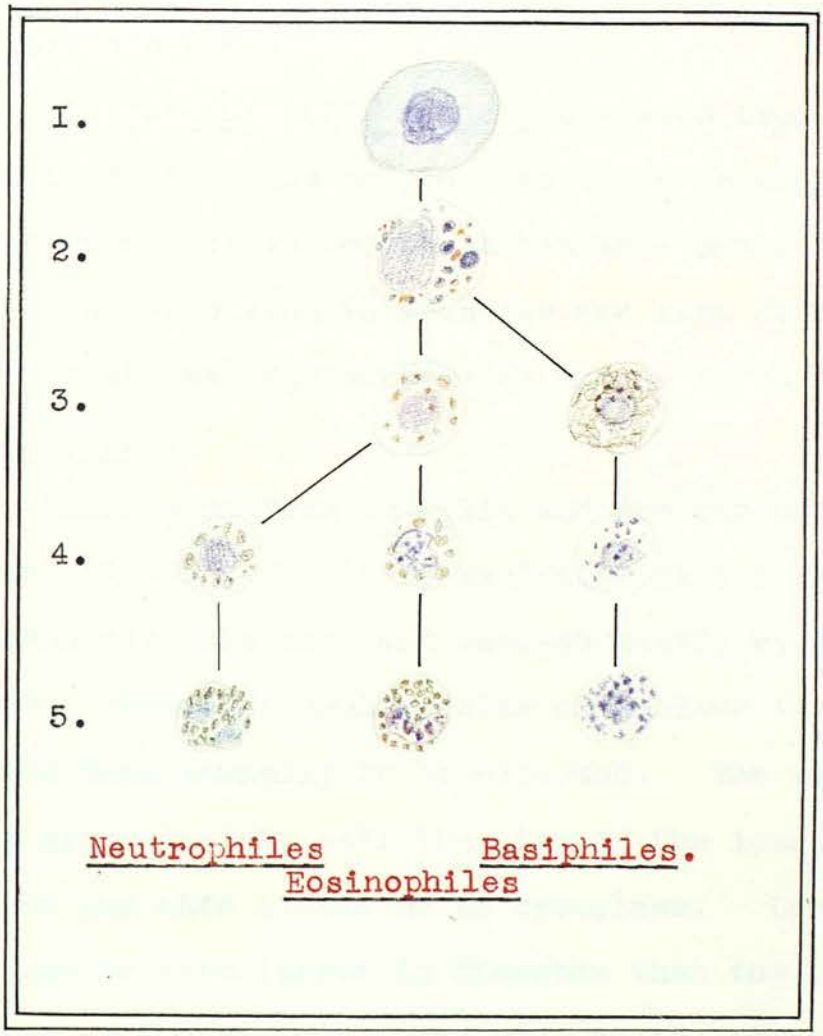
The granules are closely packed, and overlie the nucleus which is bright mid-purple in colour contrasted with the bluish-purple of the nucleus of the neutrophile. The oxychromatin is prominent in the eosinophile and deficient in the neutrophile, and the former cell usually has a bi-lobed nucleus.

Occasionally cells half way between the typical neutrophile and eosinophile are noted with rounded or elongated neutral tinted granules - usually non-refractile - the nucleus is monolobular. This apparently represents a connecting cell between them and their common ancestor the pre-myelocyte - although it has more characteristics of the neutrophile than the eosinophile.

The Basiphile (9u)

The granules of this cell are characteristic and typical of the basiphile granulocyte series of leucocytes in all animal bloods. Normally they fill two-thirds to three-quarters of the cell and overlie the monolobular nucleus, they are of irregular size, appear to fuse together and stain all shades of deep purple colour; where the granules are most compact the cell appears jet black purple in colour, but where a few can be seen separated

The Development of Avian Granulocytes.



I. Myeloblast.
2. Premyelocyte.
3. Pre-neutro-eosinophile, and pre-basiphile myelocytes.
4. Myelocytes.
5. Adult granulocytes.

they appear almost neutral tinted. The nucleus is also pale purple and with few obvious chromatic characters.

The basiphile myelocyte has fewer granules, these are scattered throughout the cell and the myelocytic character of the nucleus is obvious.

The pre-basiphile myelocyte - seen typically in haemocytoblastosis - has no granules in the cytoplasm which appears to represent an amorphous matrix - neutral or purple coloured. A few basiphile granules are seen over the nucleus, particularly at its edge where they appear to be secreted.

The Lymphocytes

These vary from 6u - llu and are the predominant cell in normal fowl blood. The large varieties have a typical lymphatic-type nucleus with the oxy- and basi-chromatin well differentiated, but in some there is a smaller size of nucleus in the largest lymphocytes than normally to be expected. The smallest lymphocytes are approximately half the size of the long axis of an erythrocyte and have little or no cytoplasm. Large lymphocytes are as large or even larger in diameter than the long axis of the erythrocyte. The nucleus loses most of its oxychromatin and is pyknotic - in many respects it is very like that of the thrombocytes, but usually round whereas the latter is frequently oval.

Vacuoles are common to all types of lymphocytes, as are

azur granules - sometimes the latter rest inside the former.

"Turk-type cytoplasm is also common - but in some cells only the periphery of the cytoplasm is deeply basic.

The Monocytes

Monocytes are not common in the blood of the fowl, possibly related to the absence in the hen of lymph glands or other large collections of lymphoid tissue generally.

Approximately 13u in diameter, there is seldom any confusion between the monocyte and lymphocyte which is contrary to the experience of bovine blood. Such easy differentiation is due to the character of the nuclei concerned, because whereas that of the large lymphocyte is typically "hillocky" or blobby in appearance, due to the well defined oxy- and basi-chromatic areas, the nucleus of the monocyte is flat and of a linear character. Nucleoli (plasmosomes) are not uncommon and in the cytoplasm azur points and vacuoles can be noted. "Turk-type cytoplasm occurs, more especially in disease conditions, but normally it is a paler blue, sometimes with eosinophilic areas scattered throughout. This unusual effect gives it a polychromasic appearance which also occurs in some of the lymphocytes.

The Pre-myelocyte

This large characteristic cell (10u - 15u) is only seen in health in chicks a few hours old, but it is common in many diseases stimulating the bone marrow, such as fowl paralysis,

chronic coccidiosis, tuberculosis and also in haemocytoblastosis.

The nucleus is always monolobular with few differential characters, but the cytoplasm contains numbers of basic, eosinophilic and neutrophilic granules. The basiphile granules are the largest and therefore contrast strongly with the bright golden colour of those tinted eosinophile.

An examination of many such cells shows that sometimes the one and sometimes the other type of granule predominate, so that it is easy to visualise this cell as the direct precursor of each of the granulocytes (myelocytes).

The Thrombocytes

The thrombocytes are smaller (5u x 10u) than the erythrocytes (7u x 12u) but of similar though not identical shape, being fusiform rather than oval.

Thrombocytes possess several important characteristics. The nucleus is like that of the small lymphocyte, rather pyknotic, but more oval and frequently eccentric in position. If the cell is visualised as an involuted semi-degenerate erythrocyte and if it is agreed that the nucleus of the red cell is supported by a reticular framework it is perhaps easy to understand why the thrombocyte nucleus readily becomes displaced from its central position. At the same time, although thromboplastid formation does occur, extrusion of the nucleus is not as common as in the red cell, therefore the reticular degeneration accompanying

normal thrombocytopoiesis cannot be as advanced as in erythroblastosis.

The cytoplasm stains only faintly - very pale dull blue colour - and is frequently vacuolated. Azur points or " dots" are common and in many instances a few such red granules may be seen clumped together within a vacuole. The majority of thrombocytes have both azur granules and vacuoles, but some cells have only the one or the other and a few have neither of these features.

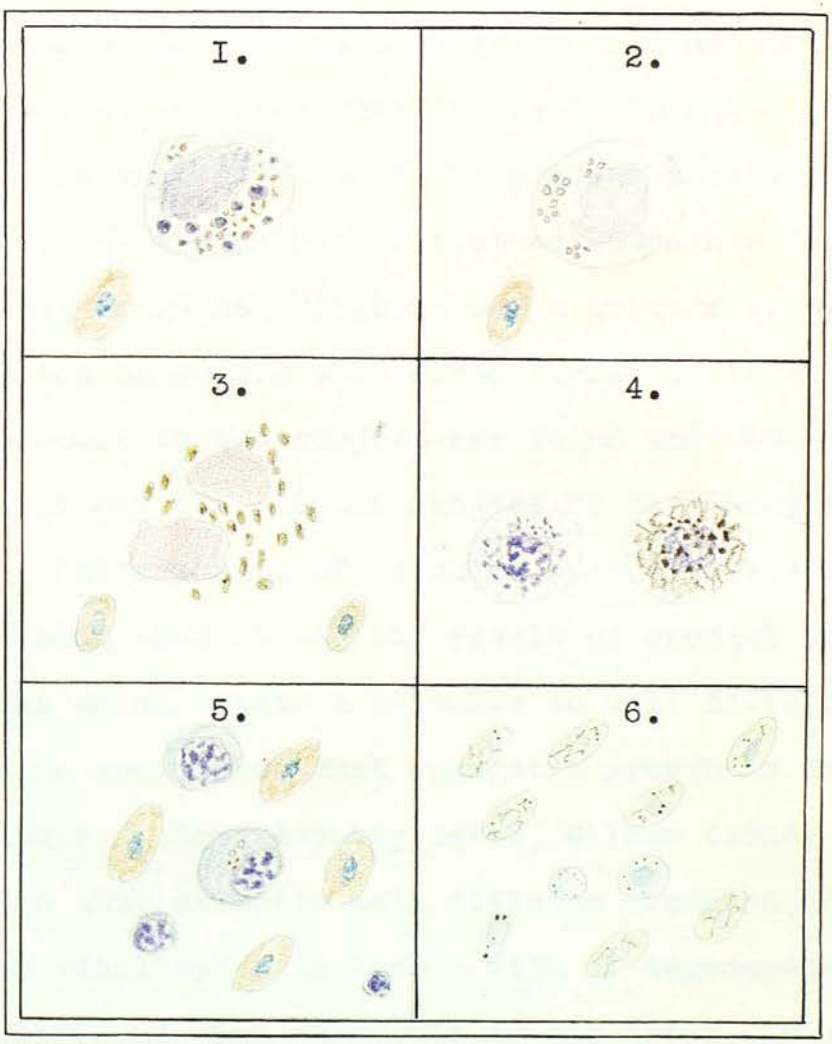
Under stress of production numbers of thrombocytes lose their characteristic fusiform appearance and thereafter all shapes may be noted, including round ones presumably related to the round erythrocytes also observed when the bone marrow is stimulated abnormally.

THE POLYNUCLEAR COUNT

It is possible to stain the nuclei of the neutrophiles leaving the granules unstained, this allows a polynuclear count to be made but it is not very satisfactory for clinical purposes. The bi-lobed neutrophile is predominant in the domestic hen and the average weighted mean index is 1.94 contrasted with 1.58 for turkeys, thus:-

	Domestic Hen	Turkey
Class I	26	42
Class II	54	58
Class III	20	0
Weighted Mean Index	1.94	1.58

Blood Cells of the Domestic Hen.



I. Premyelocyte. 2. Monocyte.
3. Ruptured neutrophile.
4. Basiphile myelocyte, and also pre-basiphile myelocyte.
5. Lymphocytes - all sizes.
6. Vital staining of red cells, lymphocytes and one thrombocyte.

AMITOSIS AND THROMBOCYTOPOIESIS

Unfortunately some textbooks refer to the blood platelet as a thrombocyte, whereas of course according to the opinion of many authorities based on Wright's work (1910) the blood platelet is derived as a fragment from the marrow megakaryocyte, and therefore in itself is not a cell.

In that portion of the present thesis relative to Leukaemia, the writer has criticised McGowan's work on pigs and poultry but would here like to pay a tribute to his valuable contribution on amitosis in blood formation in the domestic hen. Few references to the subject are found and fewer still to any relation it may have to the subject of thrombocytopoiesis.

Patterson (1907) studied amitosis relative to pigeons and concluded that it was the result of special physiological conditions which create a stimulus to cell division, Maximow (1908) also considered that energetic growth is the stimulus for amitosis. Considerably later, Wilson (1925) came to the conclusion that amitotic cell division occurred in cells of "weakened vitality" - in fact a sign of degeneration. McGowan (1925) concludes that "Amitosis is the chief, if not the only, mode of division and multiplication of the stem cells of the blood." He also states that "Amitosis would appear therefore to be a mechanism, whereby cell shape and size can be altered without alteration of the intrinsic nature of the cell." McGowan

therefore concludes that amitosis is responsible for the appearance of anisocytosis and poikilocytosis in the circulating blood, particularly so in leucotic fowls where an irritative hyperplasia of the marrow exists. He also attributes the same type of cell division as being responsible for the formation of "Fusiform cells" in the hen - thrombocytopoiesis - associating this process with amitotic division of the erythrogenic stem cells.

It will be seen that this fact alone is not in agreement with McGowan's statements, for in such a case the cell division will have "altered the intrinsic nature of the cell" from a respiratory function to one associated with blood clotting.

As far as can be ascertained, this is the first reference in the literature to an association between erythrocytopoiesis and thrombocyte function, therefore McGowan deserves every credit for what may appear to many to be a somewhat remarkable conclusion.

In the present investigation, the writer has acquired certain morphological data which tends to confirm McGowan's claim that the avian thrombocyte is related to erythropoiesis. However, although there appears little doubt that the thrombocyte is a cell of the erythrocyte series undergoing involution, no evidence has been acquired which suggests that thrombocytopoiesis normally follows amitotic cell division.

In the opinion of the writer, the thrombocyte is an erythrocyte in which the process of haemoglobinisation of the cell does not proceed to maturity. Maturation of the cell as a member of the erythrocyte series fails, and as a result of degenerative phenomena the cytoplasm loses its characteristic polychromasic-eosinophilic tinctorial property to stain faintly basiphilic.

A characteristic feature of the thrombocyte is its full oval-round nucleus which contrasts clearly with the contracted and more narrow nucleus of the erythrocyte. This feature is noteworthy in the early stages of thrombopoiesis immediately following the departure from the true erythroblast characters. It is this feature of the nucleus which causes some confusion between the thrombocyte and certain of the small lymphocytes which have nuclei of similar shape. Considerable experience may be necessary to differentiate the two cells and it is this point in computing the differential count which has caused the data of certain workers to show such wide variations from the average normal figures.

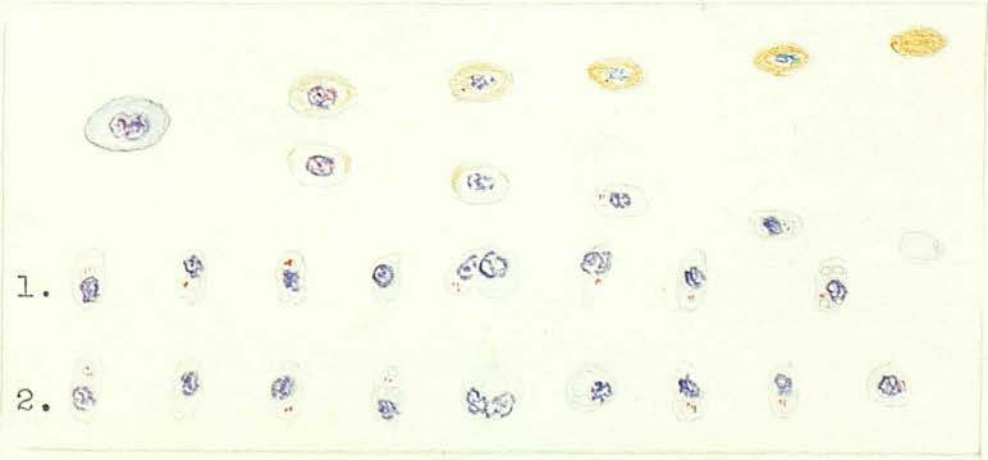
The majority of thrombocytes show vacuolation of the cytoplasm, eccentricity of position of the nucleus and one or more intra-cytoplasmic "granules." These are frequently found within a vacuole and assume azur tints after the use of Giemsa stains. No evidence was obtained to show that these granules were remnants of haemoglobin as suggested by certain authors,

and certainly from their colour in stained preparations they are more closely related to the azur cytoplasmic inclusions of the lymphocytes than to the unaltered haemoglobin of erythrocytes

Contrasted with the circulating red cells of the blood, the nuclei of the thrombocytes are more oval associated with their general fusiform shape which differs from that of a typical erythrocyte. In the same way that there are found in the blood-stream - notably under stress of production - round instead of oval erythrocytes, so one finds round thrombocytes. In the turkey rounded thrombocytes are very common in Blackhead, in which disease the vacuoles of the thrombocytes are large and the azur granules deficient.

The accompanying diagrams drawn from numerous blood films depict some of the features described.

THROMBOCYTE FORMATION



Believed by the writer to occur through involution of those erythrocytes which fail to become haemoglobiniferous.

1. and 2. represent various types of thrombocytes, seen in the blood of poultry.

On rare occasions a thromboplastid will be seen in which the nucleus has disappeared, probably by extrusion as seen in the formation of erythroplastids. In supravitally stained films the thrombocytes show basic staining inclusion spots allied to those in the erythrocytes and quite dissimilar from the fine basic points of the lymphocytes.

If films stained brilliant-cresyl-blue are counter-stained with Pappenheim's panoptic method, the azur granules of the thrombocytes contrasting with the pale blue cytoplasm resemble greatly the blood platelets of mammals. The function of the thrombocyte relative to blood clotting is believed to be similar to that of the blood platelet.

All stages of the erythroblast-thrombocyte series can be detected in smears prepared from the bone marrow. Thrombocytes with double nuclei are rare, but afford further evidence of their association morphologically with erythrocytes in which the same function can be noted.

THE NORMAL BLOOD PICTURE OF THE DOMESTIC HEN

Literature

During the past 20 years the literature on avian haematology has increased considerably and there is now available data relative to a number of normal counts which may be considered satisfactory.

In general, of course, there is no standard blood count for the domestic hen unless age is considered as well as health and sex, for although the latter has little effect on the differential leucocyte count it does affect the haemoglobin percentage and total red cell count.

Standard counts based on an average of the results obtained by the following workers have been prepared and contrasted with those of the present investigation:- Barcroft (1929), Bayon (1929), Bedson and Knight (1924), Biely and Palmer (1935), Blain (1928), Blakemore (1934-35), Bronkhorst and Hall (1935), Burnett (1908), Cook and Dearstyne (1934), Doyle (1929), Forkner (1929), Goodall (1909), Harmon (1936) Hayden (1927), Hayden and Fish (1928), Hofmeister (1934), Holmes *et alia* (1933), Kaupp (1922), Kelly and Dearstyne (1935), Kozma (1928), Kyes (1929), Seagar (1933), Ward and Gallacher (1920). The results for adult birds are as follows:-

	Various Authors	The writer
Red blood cells	2,945,000	3,174,000
Haemoglobin	74%	74%
White blood cells	24,490	27,000
Neutrophiles	29.6	20.3
Eosinophiles	4.8	3.0
Basiphiles	2.8	2.4
Lymphocytes	59.3	73.9
Monocytes	3.5	0.4
LL:SL ratio	-	1 : 5.3

Haemoglobin

The percentage of haemoglobin recorded above on the Tallquist scale corresponds to 10.75 grammes/100 c.c. Holmes et alia (1935) in some carefully recorded experiments showed that at 21 days after birth the amounts of haemoglobin were 9.6 grammes for cockerels and 9.3 grammes for pullets. At 12 weeks these figures had risen respectively to 10.1 and 9.7 grammes/100 c.c. Such a distinction may not exist if birds are reared intensively but it appears clear, no matter what type of management, the percentage at birth will fall considerably during the first two weeks of life. After this, a steady rise to 70% - 80% at maturity should be recorded, i.e. the range throughout life is 8 - 11mg HB/100 c.c. - highest in cocks, less in capons and lower still in pullets. (Harmon 1936).

Erythrocytes

The number of red cells recorded at hatching is always considerably lower than in the mature birds - the respective figures are approximately 2,000,000 to 3,500,000 erythrocytes/c.mm. Variations recorded in the literature are probably associated with conditions of management, but it seems probable few adult birds in health have less than 3,000,000 red cells/c.mm. of blood. Bronkhorst and Hall have investigated the relationship between high and low hatching hens but failed to find any significant difference in their erythrocyte contents.

Leucocytes

Contrasted with mammals, the domestic hen, and indeed poultry and pigeons generally, have a normal high leucocyte count from 25,000 - 30,000 cells/c.mm. About ten years ago considerable confusion arose because Kyes (1929) stated that the numbers in poultry were little different from those of mammals 8 - 13,000; Burnett's average was also low at 17,921 leucocytes /c.mm. - especially contrasted with the 38,000 of Hayden,(1927.) Since that time various refinements in technique have been established and the normal figure is approximately 27,000. It is interesting to note that Goodall carefully arrived at an almost identical figure (25,000) nearly twenty years ago.

It seems clear the number of leucocytes in chicks in-shell is extremely low, higher at birth (8-9,000) and showing

a triple rise as maturity is reached.

Differential Count

This too cannot be computed from the literature because of errors of technique, as the following figures show wide variations not compatible with health:-

Author	N.	E.	B.	L.	M.
Blain	49.3	8.66	3.63	32.76	5.65
Seagar	34	5	2	54	5
Dukes	23	5	2	64	5
Hayden and Fish	10.3	5.2	1.6	81.4	1.54

As a result of my own examinations of poultry bloods, now several hundred in number both in conditions of health and disease, it can be stated that the neutrophile percentage is always definitely low in the domestic hen after 3-4 weeks of age. The extreme limits compatible with health are from 10% - 30%, but of course at birth a relative neutrophilia up to 80% is normal.

No doubt numbers of workers have confused the smallest lymphocytes with the thrombocytes, so allowing the percentage of neutrophiles recorded to be higher than was actually the case.

Although the percentage of eosinophiles is slightly higher than in man there is a marked increase in the basiphiles which are frequently present in equivalent numbers.

The lymphocytes predominate, notably the small varieties, but these are never as small as some pyknotic varieties devoid of

cytoplasm seen in diseased birds - coccidiosis and fowl paralysis. The large lymphocyte-small lymphocyte ratio is wide - as 1:5 or as 1:6, and in the opinion of the writer is a valuable guide as to the health of a bird.

Careful examination of the nuclei of the large mononuclears will disclose few true monocytes and in this respect the writer's data therefore agrees with that of Hayden and Fish (1928).

An average differential count in a normal healthy mature domestic fowl reads approximately:-

N.	E.	B.	L.L.	S.L.	T.L.	M.
20	3.0	2.5	12	62	74	.5

THE GUTTADIAPHOT

Schilling's (1929a) well known Guttadiaphot blood testing papers can be used satisfactorily on most of the lower animals but they are useless for assessing the normality of avian blood, for the following advanced Guttadiaphot reaction will be given by any normal hen:-

I = (a) + b + (e)

II = b + e

III = b + d + e

This failure was recorded by the writer in 1931, but since that time an additional point of interest concerns the development of a "halo" which surrounds the drop of blood on the Tallquist absorbent papers when used for poultry. Its appearance is striking, especially if viewed by holding the slip direct to the light - and it is a specific feature for normal avian blood, although a similar halo occurs when using the blood of cattle suffering from secondary anaemias, such as that accompanying tuberculosis and Johne's disease.

In 1931 the writer was not using the Tallquist method for estimating haemoglobin, therefore the association between this halo and Schilling's "ring" was not known. The Tallquist reaction is typical and would be classed as advanced corona formation if the Guttadiaphot papers were substituted for that of Tallquist.

A survey of the accompanying Table shows that although the degree of corona formation is not directly proportional to the percentage of haemoglobin a rough relationship between them exists as follows:-

Tallquist % Scale	Corona Formation W.M.Index
40 - 50	3.8
51 - 60	2.6
61 - 70	2.5
71 - 80	1.8
81 - 90	2.2

Exceptionally wide halos are seen accompanying the anaemias but as the haemoglobin percentage rises so the size of the halo progressively diminishes, but not in direct proportion. At all stages, there are birds whose blood shows fairly advanced corona formation independent of the haemoglobin percentage.

The width of the halo, therefore, is more probably related to the viscosity of the blood plasma rather than to any simple deficiency of haemoglobin *per se*.

Mammalian bloods only show similar corona formation in disease - that in healthy poultry is a specific phenomenon for *Aves*.

TALLQUIST CORONA FORMATION

HB%		Extent of halo
45	=	+++++
45	=	+++++
45	=	++++
46	=	+++++
47	=	++++
49	=	+++
50	=	+++
50	=	+
55	=	+++
55	=	++++
55	=	+++
58	=	++
60	=	+++
60	=	++
60	=	++
60	=	++
60	=	+
60	=	++++
62	=	+++
62	=	+++
62	=	++++
63	=	++++
64	=	++
65	=	+
65	=	+
65	=	+
65	=	+++
68	=	++++
68	=	++
68	=	+++
70	=	++
70	=	+
70	=	+++
72	=	+
72	=	++
72	=	++
74	=	+++
75	=	++
76	=	++
76	=	+
77	=	++
78	=	++
78	=	+
78	=	++
78	=	+
78	=	+++
80	=	++
80	=	++
80	=	+
80	=	+++
80	=	½+
84	=	+++
85	=	+
85	=	++++
90	=	+
90	=	++
120	=	½+

THE BLOOD PICTURE AT BIRTH IN THE CHICK

In the day old calf there are marked changes in the blood picture contrasted with that of the adult cow, but in the case of the day old chick many more marked features are observed. In both, a relative neutrophilia is a constant finding but in the chick the erythroblastosis picture of the foetus persists prominently. Indeed, the erythrocytes so dominate the field few white cells are noted - the total leucocyte count although only 8,000 per c.mm. is almost double that seen in chicks "in-shell."

At birth, erythropoiesis is extremely active and all forms of erythrocytes and their precursors may be found in the blood-stream - red cells representative of regenerative and degenerative forms being noteworthy. It is interesting also to observe that the erythrocyte at this stage never fully matures, because the nucleus remains purple coloured and does not assume the blue tints of the older red cells seen in healthy adult poultry.

Erythroblasts, both basic and polychromasic, are common, some of the former showing typical mitotic figures. Large numbers of the immature erythrocytes degenerate, so that nuclear remnants and erythroplastids are common. However, a number of others do exhibit pyknotic nuclei, frequently with hyperchromatic cytoplasm.

Micro-blasts (haemocytoblasts - Emmel) are common, as are certain specially small thrombocytes but there is probably a direct genetic link between the two cells, therefore their numerical relationship is to be expected.

The leucopenia observed is characterised by a marked relative predominance of the neutrophiles, many of which are small but some are larger than normal, i.e. pre-neutrophilic myelocytes. Their granules are more round than typically rod shaped and they stain more basically than usual in avian neutrophiles. The basiphile granulocytes are often immature with an incomplete complement of granules and a neutrophilic cytoplasmic groundwork. A few birds also show true pre-myelocytes in blood films, but this is not common to all day old chicks.

"Turk-type cytoplasm is common in the few lymphocytes and monocytes noted.

Although it is probably quite correct to consider this type of blood picture normal and merely representing a physiological transition stage from that of the embryo chick in-shell to one typical of the adult bird, the features noted are identical with those considered by Emmel as "haemocytoblastosis."

A number of chicks actually emerging from the shell, many of which would have been unable to break through of their own accord and would probably have died, have also been examined. These show the same features described for day old chicks, but

some are more accentuated.

The haemoglobin percentage, total leucocyte count and relative neutrophilia are lower than normal at birth, indeed the deficiency of leucocytes is so acute it is impossible to find even 100 cells for counting purposes. In many day old pigeons it is impossible to find even one normal leucocyte in a film - all the cells being of the erythrocyte series, but in the domestic hen the leucopenia is never quite so complete.

Chicks stuck in-shell show considerable variation in the size of the red cells - an anisocytosis more marked than that in healthy young chicks, they also show numerous neutrophiles ruptured due no doubt to their defective construction having been disclosed by the preparation of the film.

Poikilocytosis is not common and punctate basiphilia never seen at all.

The accompanying Table shows the chief features of the blood picture in chicks contrasted with that of older birds.

The figures for individual chicks vary somewhat during the first day of life, no doubt due to the fact that chicks purchased as day old may in fact be early or late hatched, i.e. a few hours or even 36 hours old, for in the present day type of mammoth incubator the hatching process is not disturbed until the end of the 21st. day, and some chicks pip on the 20th. day of incubation.

NORMAL BLOOD PICTURE OF THE DOMESTIC HEN

	Chicks in-shell	Day old chicks	Chicks 10 days old	Mature pullets
White blood cells	4,275	8,400	9,433	27,000
Haemoglobin %	61	73	66	74
RBC's (000's)	2,250	2,500 X	2,700	3,174
Early granulocytes	++	+	-	-
Neutrophiles	51.6	64.6	52.0	20.3
Eosinophiles	3.2	1.4	2.3	3.0
Basiphiles	6.6	3.1	6.4	2.4
Large lymphocytes	8.4	4.4	5.8	11.7
Medium lymphocytes	17.0	13.8	15.3	-
Small lymphocytes	10.9	11.6	17.6	62.2
Total lymphocytes	36.0	29.8	38.7	73.9
Monocytes	2.4	0.9	0.2	0.4
LL:SL ratio	.87:1.0	.61:1.0	.53:1.0	.19:1.0
Erythroblasts	++++	+++	+	-
Erythroplastids	+++	+++	+	-
Anisocytosis	+++	++	+	+

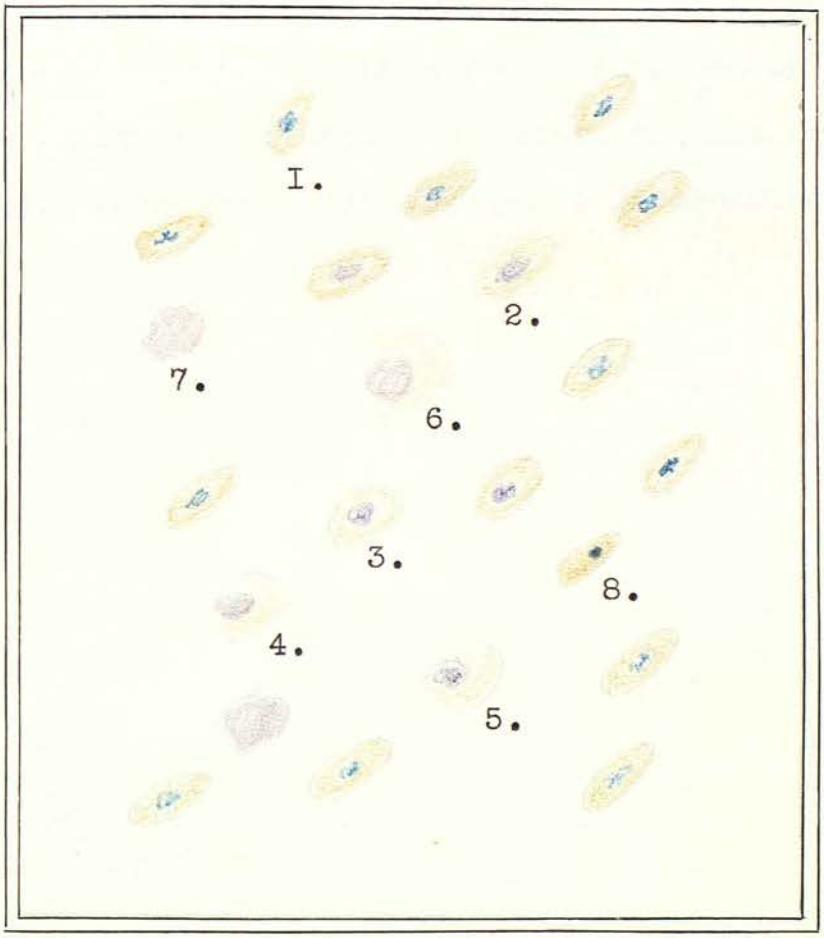
X Recorded in the literature - Biely and Palmer (1935).

THE INFLUENCE OF BROODINESS ON THE BLOOD PICTURE OF THE FOWL

It is generally suggested that broodiness is associated with a physiological disturbance in endocrine function, therefore it is of interest to speculate whether the differential count is altered during broodiness due to the influence of such a ductless gland as the thyroid.

Somewhat contrary to expectation, there is no lymphocytosis of thyroid origin - the neutrophiles are increased, and there may be a reversed basiphile-eosinophilic dominance - but the LL:SL ratio is wide (1:11) and the total WBC count is normal. The erythrocytes are usually mature, but appear to degenerate rather more rapidly than usual, to leave - after intravascular haemolysis has taken place - relatively large numbers of nuclear remnants in the circulation. Such erythronoclasia is not specific for broodiness and is depicted by the finding of one nuclear remnant to every two leucocytes, i.e. there will be approximately three nuclear remnants in every two "fields" examined. Polychromasic erythroblasts are seen very occasionally, and although erythrocytes show nuclei with notches, a number are pyknotic. A typical example of the differential count of a broody hen is:-

Nuclear Remnant Formation in the Hen.



I. Normal erythrocyte.
2,3,4,5 and 6. Successive stages leading to nuclear remnant formation.
7. Nuclear remnant. 8. Pyknotic R.B.C.

Ref:	P.	E.	B.	L.L.	S.L.	T.L.	M.	WBC's
220	31	2	4	5.1	57.9	63	0	27,000
Normal fowl	20.3	3	2.4	11.7	62.2	73.9	.4	27,000

Gunther (1935) in a dissertation at Leipzig, showed that an enormous number of degenerate red blood cells were present during the moult in poultry. The cause of this is possibly closely related to that indicated above in broodiness.

EOSINOPHILIA AND BASIPHILIA

Literature

Piney and Wyard (1938) consider that eosinophilia in man may be a response of the bone marrow to a non-bacterial toxin, they state that very high figures are found in trichiniasis and that eosinophilia is an accompaniment of infestations by intestinal worms (Filariasis), hydatid cysts and of certain skin diseases. The number of cells generally being higher the larger the area of skin involved.

Whitby and Britton (1935) go further, assuming that an eosinophilia (over 4%) is of definite diagnostic importance in infections with tapeworms, threadworms, ascaris, etc. These authors confirm that eosinophilia may accompany certain allergic states (notably bronchial asthma, hay fever and urticaria), and consider that it is often present in tuberculin reactions. Concerning the increase of eosinophile granulocytes in skin disorders they consider it fairly constant in pemphigus, dermatitis herpetiformis, scabies, psoriasis, eczema and prurigo. Its significance as a post-infective phenomenon is also mentioned as well as that seen to accompany the feeding of raw liver to pernicious anaemia patients. (Whitby 1928). Biggart (1932) has pointed out that the production of eosinophiles may be a protective response against a foreign protein.

McCormick considers the granules arranged in concentric fashion round the atrosphere, and believes that the diseases in which eosinophilia occurs are grave and mostly of long duration. The cells are called from the blood into the tissues by a foreign protein substance which, in his opinion, has been formed by the autolysis of the skin caused by the skin lesion. He mentions Ehrlich's conception of the process, i.e. a pathological reaction to a native protein being broken up inside the body - the result of epithelial or cellular destruction.

Maas (1933) experimenting on the production of eosinophilia in pigs associated with trichinosis found it was maximal at about three weeks after an infestation and roughly proportional to the intensity of the infestation. Eichler reached a similar conclusion in 1930 respecting fluke invasions of cattle, whilst Vallilo (1909) found an increase in eosinophiles in sclerostomiasis. Joest (1908) recorded eosinophilia in chronic multiple intestitial hepatitis, echinococcosis and distomatosis with no increase in these cells in tuberculosis and melanosis. Ebhard (1909) believed eosinophilia to be a response to painful diseases, ascribing the condition to chemotactic properties of parasites.

Newmann (1928) has suggested that the granules of the eosinophile leucocyte are of a fatty nature, probably containing a highly organised oxidase, giving rise to several peroxidases.

Haematology

A study of 82 animals in the present series of cases showing more than 5% of eosinophiles or basiphiles in poultry and over 10% eosinophiles in cattle has been made. They were distributed as follows:-

	Cases
Eosinophilia in the Domestic Hen	30
Basiphilia in the Domestic Hen	26
Eosinophilia in cattle	26

Eosinophilia in cattle

Although a series of six cases in cattle suffering from chronic bronchitis averaged 12.5% eosinophiles (which appeared to confirm the findings regarding asthma in man) it should be noted that percentages considerably higher were found in a number of healthy cattle.

The average percentage in normal heifers and dairy cows was about 16.5%, so that 11.5% in a tuberculin reacting cow and a similar figure in a case of early tuberculosis is probably not of importance. On the other hand, it is significant that 10 heifers reacting to the abortion test and negative to tuberculosis gave an average percentage of 21.8%

Fraser (1930) suggested that the percentage of eosinophiles in cattle rose with age, and although this is not true it is important to realise that as tuberculosis and abortion

are so widespread in this country, the older an animal, the greater chance it has of becoming infected, and certain stages of both diseases are apparently capable of causing eosinophilia. Even so, this does not explain the high eosinophilia of a number of bovines tubercle and abortion-free. In fact, this investigation gives few clues as to the true nature of eosinophilia of bovines, but it is probable the bone marrow is stimulated by a variety of causes.

Eosinophilia of the Domestic Hen

The percentage of eosinophiles in healthy fowls is always less than 5%, so that the following percentages in the 30 birds examined are of interest:-

	% eosinophiles
Healthy day-old chick	6.0
Fowl paralysis	6.3
Following liver extract injections	8.8
Infectious catarrh	9.0
Coccidiosis	9.0
Tapeworm infestations	10.5
Gapeworm infestations	12.0
Fowl paralysis iritis with coccidiosis	12.8
"Normal" hens	17.0
Tapeworms and ascaridia	31.7

It should be noted that the so-called normal hens were not examined specifically for intestinal parasites, and it is also necessary to point out that none of the above results were specific for any one disease group - other animals similarly affected showing no eosinophilia, thus:-

Worm Infestations	% eosinophiles
Davainea ++ Capillaria + Coccidia +	5.0
Davainea ++ Railletina +	15.7
Davainea +++	5.7
Davainea +++ Coccidia +++	0.0
Amoebotaenia +++	2.0
Davainea ++++ Ascaridia +	4.3
Heterakis +++	0.7
Heterakis +++ Ascaridia ++	0.0
Davainea ++++ Ascaridia ++	31.7

It is clear that an eosinophilia in poultry cannot be co-related with the degree of parasitosis present.

There appears only one factor common to the recognised condition in animals in which an eosinophilia is to be expected, namely, an irritation of epithelium or of tissues of epithelial origin, e.g. skin, bronchial mucosa, intestinal epithelium, liver, etc. This conforms to the belief of Ehrlich that eosinophilia is a result of epithelial or cellular destruction,

but it is far from clear whether it is directly related to the absorption of products of cell destruction acting as a stimulus to the bone marrow.

There is a considerable amount of evidence to show that eosinopenia is frequently related to certain pyogenic infections in animals, but there is little to show that a post-infective eosinophilia occurs comparable with that in man.

Basiphilia in the Domestic Hen

The highest percentage recorded (48%) was noted in a case of severe intestinal toxaemia, 34% was seen in a chick suffering from advanced amyloid degeneration of both kidneys in association with coccidiosis, but in a number of uncomplicated cases of this latter disease 6% of basiphiles were recorded, whereas the average for all cases of coccidiosis was only 3.34%.

Perhaps the most interesting feature was the relatively high percentage found in chilled chicks (17.7%) and in certain chicks in-shell or only one or two days old - 8%.

In some cases of fowl paralysis and iritis 6% - 7% were noted - figures also typical for certain cases of coccidiosis and davainea infestations. This may have been related to the findings of Kennedy and Law (1935) who found the proportion of basiphiles to rise - almost proportional to the dose - after the administration of embryonated Toxascaris eggs to three months old fox cubs.

Following the tuberculin testing of poultry, three birds examined showed a rise of basiphiles to 5% - 6% - this may be of practical clinical importance in the elimination of false tuberculin reactions, in which diseased birds fail to show any recognisable response to intra-wattle injections.

Apart from this question of basiphilia relative to tuberculosis, the only feature common to all the other types was that of stagnation of the intestinal contents, which is presumably followed by the absorption of toxins which stimulate basiphile production.

AVIAN TUBERCULOSIS

Clinical Notes

In the domestic fowl during life, tuberculosis is characterised by anaemia, wasting, diarrhoea and sometimes by blindness. Occasionally, a hen in absolutely fat condition will die from internal haemorrhage following rupture of a tuberculous liver.

The average age of affected hens is 14 months, but tuberculosis in its typical form generally attacks hens about two years old.

In 90% of cases, the post-mortem findings show both the liver and spleen to be affected. Numerous tubercles are found, sometimes coalescing to form large nodules. In 40% of cases, there are lesions involving either the intestines or bone marrow, although the latter are not always discovered because they are not sought for during routine examinations. As a contrast to tuberculosis in mammalian farm stock, the lungs rarely become involved, for the natural method of infection is by ingestion and not inhalation.

Haematology

The blood picture of tuberculosis in poultry is particularly interesting, and in order to observe the changes clearly, a cockerel was injected intra-muscularly with spleen emulsion from a hen found suffering from the disease in an advanced form. This cockerel had been in the writer's possession

for over 12 months, during which time he was tuberculin tested and agglutination (B.W.D.) tested, and found negative to both; also, having been housed on a wire floor, there was little chance that parasites were present in the intestines. Therefore, the blood changes recorded may be considered due entirely to tubercle infection.

Following the inoculation of the cockerel with diseased material, a tuberculin test was applied after eight days, but it gave an indefinite reaction. A second applied eleven days later was markedly positive - 20 days after original injection.

The accompanying table shows the blood changes recorded for this bird - the last three items refer to three birds suffering from advanced clinical tuberculosis, confirmed by post-mortem examinations.

TUBERCULOSIS

Date or Ref:	Hb.	WBCs	P.	E.	B.	T.L.	Mono.
19/8	80	24,500	21	.3	4.7	73.3	.7
25/8	80	-	20.6	1.7	1.7	75.3	.7
28/8	80	21,000	24.7	2.0	5.7	66.6	1.0
2/9	73	27,000	23.0	.3	2.3	73.4	1.0
8/9	87	17,800	20.7	1.6	3.3	73.7	.7
9/9	84	28,000	50.7	1.7	1.7	45.6	.3
4/10	77	32,000	20.6	1.0	2.3	75.4	.7
11/10	74	15,000	38.0	2.0	1.0	59.0	0
173	60	38,500	40.25	3.25	5.0	51.5	0
221	60	30,000	54.0	2.3	6.0	37.7	0
126	46	45,000	62.5	1.5	1.0	35.0	0

Once tuberculosis has become fully established - as diagnosed by the appearance of a positive tuberculin test - several important blood changes occur. The haemoglobin content falls progressively with an extension of the disease, whilst the associated anaemia although not sufficiently severe to introduce erythroblasts into the circulation, is characterised by the appearance of erythrocytes with notches, nuclear pyknosis, nuclear remnants and erythroplastid formation.

The total leucocyte count is somewhat irregular but in general a definite increase of white cells occurs, especially in typically advanced clinical cases.

The differential count shows changes in several types of cells, notably the polymorphs and lymphocytes. The former rise steadily, almost parallel with the disease process, until at death there are 50% or more neutrophiles present, many of which appear fragile and rupture during the preparation of the film. This neutrophilia also seems to be marked as an after-effect of the tuberculin test - when the latter is positive.

Associated with the general rise in the neutrophiles is a steady fall in the percentage of total lymphocytes, although an increase in the largest variety occurs early on in the disease, to remain higher than normal throughout the whole illness. The medium sized lymphocytes fluctuate, but decrease markedly when clinical symptoms are evident, as do the small lymphocytes. The LL:SL ratio falls continuously, always tending to become narrower

than normal, temporary remissions being followed by a falling ratio.

LYMPHOCYTE RATIO

Ref:	L.	M.	S.	LL:SL Ratio
226	10	14.3	49	1 : 3.3
252	10	22.3	43	1 : 2.5
273	10	22	34.6	1 : 2.2
289	15	33.7	24.7	1 : 1.3
297	13.7	11.7	48.3	1 : 2.8
302	11.7	15.3	18.6	1 : 1.4
341	11	20.7	43.7	1 : 2.5
346	16	22	21	1 : 1.2
173	19.7	2.7	15.3	1 : 0.8
221	27.75	10.0	13.75	1 : 0.6
126	15.25	1.25	18.5	1 : 1.2

Many medium sized as well as other lymphocytes appear vacuolated, some also show azur bodies or alternatively Türk-type cytoplasm - additional degenerative features are seen in some of the leucocytes, in which the granules are toxic or sparsely distributed throughout the cell.

The monocytes are never increased beyond the limits of health and in all advanced cases are depressed - often completely so that none are seen at all.

Neither the eosinophiles nor the basiphiles show any constant change in the differential count in tuberculosis, nor is the ratio between them a fixed one. The eosinophiles are never absent from the picture, indeed they tend to be present in increased numbers towards the end of the disease, whereas the basiphiles show percentages above normal in both early and late stages.

The blood picture therefore would appear to be a good guide as to diagnosis and prognosis in avian tuberculosis.

In the former, a combination of a sub-normal haemoglobin content, leucocytosis, neutrophilia, increase in large lymphocytes, and depression of the monocytes suggests a positive diagnosis.

In the latter, it would appear that any increase in the leucocytes - especially when affecting both the polymorphs and large lymphocytes - should be considered unfavourable, whereas a rise in the total lymphocyte percentage - notably in the medium and small types - is a favourable prognostic sign, especially if the haemoglobin percentage also increases.

FOWL POX - CONTAGIOUS EPITHELIOMA

Clinical Notes

Fifteen to twenty years ago Fowl Pox proved a scourge to the poultry industry of this country, but thanks to the initial work of Doyle and Minett of the Ministry of Agriculture and Fisheries, on preventive vaccination the number of cases seen today is comparatively small. Nevertheless, at times severe outbreaks do occur when particularly virulent strains of the causal virus appear to be implicated. The lesions in poultry consist of wart-like scabs involving the comb, face, wattles and mouth (avian diphtheria).

Pigeon pox vaccination has been used successfully in this country for more than the past ten years but the short duration of the solid immunity conferred, i.e. approximately four months, detracts from its value. For this purpose, the breasts of young healthy pigeons are scarified and inoculated with pigeon pox virus scabs (ground up and emulsified in glycerin-saline solution). The resulting lesions mature in about seven days when the scabs can be removed, dried and stored in a refrigerator until required for use. Where there is any doubt concerning the diagnosis of Fowl Pox, it is often necessary to apply to the scarified scab of a susceptible Leghorn cockerel (used because of the well-developed comb)some of the suspected material.

Blood samples have been taken from birds suffering from natural Fowl Pox, from artificially induced cases (involving the comb) and from inoculated scarified pigeons used for vaccine production purposes.

The blood picture of true Fowl Pox, in advanced cases of the disease is quite different from that seen in experimentally inoculated Leghorn cockerels, for the former shows a reduction in the percentage of neutrophiles and eosinophiles. Pre-myelocytes are seen and in addition to the stimulation of the lymphocytes (large) numbers of the smaller varieties are vacuolated.

However, in experimental cases, and also in pigeons scarified for vaccine production purposes, there is a well-marked neutrophilia, with a reduction in both the eosinophiles and basiphiles.

In all cases of pox the LL:SL ratio will be seen to be narrower than that in healthy birds. Thus:-

	P.	E.	B.	T.L.	M.
		FOWL POX			
Natural	13.6	1.4	1.3	82	1.7
Artificial	54.9	1.2	1.0	41.9	1.7
Normal birds	20.0	3.3	3.7	72.6	.4
		PIGEON POX			
Artificial	59.2	.72	1.68	37.7	.34
Normal pigeons	26.6	4.75	4.9	63.3	.35

LYMPHOCYTE RATIO

Fowl Pox	L.L.	S.L.	Ratio
Natural	23	59	1 : 2.6
Artificial	16.9	25	1 : 1.5
Normal pullet	16.3	56.3	1 : 3.5
Pigeon Pox			
Artificial	12.8	25.9	1 : 2.0
Normal pigeon	13.4	49.4	1 : 3.7

THE BLOOD PICTURE AFTER INJECTIONS OF LIVER EXTRACT

It is generally agreed that a method for the standardisation of the potency of liver extracts for use in the treatment of pernicious anaemia is required, because although a number of attempts have been made using pigeons and rabbits no really satisfactory method has been evolved. In the pigeon this is probably due to the fact that the circulating erythrocytes in health nearly all stain with varying degrees of polychromasia, therefore reticulocyte counts are too high to be of value in detecting responses on the part of the marrow following liver extract injections.

Shaw and Pritchard (1937) used pigeons aged about three weeks, but found difficulty in making satisfactory stained preparations by the dry coverslip method, a feature also noted by Vaughan, Muller and Zetzel (1930). They conclude that the reticulocyte response of pigeons to liver extract is not a function of the clinical efficiency of liver extract. On the other hand, Edmunds and his colleagues (1933) outlined a method which they considered will differentiate a potent extract from an inert one, but the reticulocyte response was not observed until about seven days after the administration of the liver. Gurd (1935) suggests a method which shows that all pigeon erythrocytes contain some reticulation, but he only computes

those which are densely reticulated. Even so, the new technique recommended by him failed to show any response in pigeons after the administration of potent liver extract - a point confirmed by Wakerlin, Bruner and Kinsman (1936).

Davidson and Gulland (1930) state that so far as they are aware there is no satisfactory means of estimating the potency of liver extract, and they are dubious whether it can ever be accomplished by testing extracts in animals artificially made anaemic. Davidson had carried out a large number of experiments using rabbits, some of which had been injected with B. welchii toxin to produce a severe anaemia associated with marked degenerative changes in the marrow. Unfortunately, these experiments did not succeed but their investigations were being continued, since they considered the matter of great practical importance.

Less attention has been given to the domestic hen as an experimental subject for such studies, therefore the writer attempted to show a relationship between the leucocytic response and the dosage of liver extract administered in place of the usual erythrocyte (reticulocyte) reaction.

Injections from $\frac{1}{2}$ to $2\frac{1}{2}$ c.c.'s of Heparin (P.D. & Co.) were given to birds of different ages. In hens, no leucocytosis occurred but after 48 hours a relative neutrophilia was seen

in association with a rise in the percentage of the other granulocytes. It was not possible to detect in panoptically stained films any marked change in the number of polychromasic erythrocytes, but the introduction of erythroblasts into the circulation was noteworthy.

In pullets not in lay the leucocytes do increase, as do the neutrophiles and eosinophiles, whereas similar birds in lay show no leucocytosis but a delayed relative neutrophilia and basiphilia occurs after 96 hours. Erythroblasts or erythroplastids are conspicuous as a response to the injections.

In young pigeons a leucocytosis is noted with little change in the differential count except an increase in the basiphiles.

Although none of these features appear constant, it is not improbable that a series of injections into birds of differing ages (notably during the growing stage when the myeloid response is most active) would reveal some which could be used for standardisation work. The granulocytes appear better suited for such comparative work, since the response of the lymphocytes and monocytes shows no features of note.

Finally, it would then be necessary to determine whether any standard blood reaction obtained was directly related to the erythrocytic activity of the liver preparation used, otherwise as a clinical test it would be valueless. In this connection, the

peculiarity of bovine blood relative to megalocytosis suggests that the calf might prove a suitable test animal in preference to the domestic hen, especially since the percentage of reticulocytes normally found is very low - often none being present.

FOWL PARALYSIS

Clinical Notes

Lymphomatosis, commonly known as Fowl Paralysis, and originally termed neuro-lymphomatosis-gallinarum, is a specific disease of the domestic hen characterised by "enlargements" of the nervous system, or by the development of "tumours" due to infiltrations by lymphoid cells. It frequently attacks pullets on the point of lay, between the ages of five and eight months, but younger birds also suffer, and neither age nor sex renders a bird immune to the disease. In acute cases, the onset is sudden, an affected bird (usually in good condition) first showing signs of improper control of the legs or wings. It may limp or hop, or "drop" a wing, but within 48 hours a severe paralysis of the limb usually is evident, and the associated flight or perching reflexes are lost. The bird is often unable to move far, quickly loses flesh, gets "soiled" behind, and at this stage is usually killed by the farmer. The symptoms vary with the part affected, but a dropped wing or paralysed leg is most common, as is blindness associated with paralysis and depigmentation of the iris.

Fowl paralysis is believed to have been described originally in 1907 by Marek of Hungary, but Pappenheimer, Dunn and Cone published a fuller account of its pathology (as encountered in America) in 1929, although Kaupp previously mentioned its existence in that country in 1921. Since that

time, it has been recognised in many of the civilised countries of the world, separated as far apart as Germany (Dobberstein and Haupt, 1927), South Africa (Thomas, 1928), Japan (Emito and Miyamoto, 1930) and Italy (Vianello, 1933).

Galloway (1929) was the first to report its occurrence in this country, and during the past eight years it has been studied extensively by numerous agricultural and veterinary workers. The history, symptoms, post-mortem findings and general pathology of fowl paralysis appear to be well understood, but the evidence regarding its causation and infectivity is still in dispute.

Pappenheimer and his colleagues termed the disease "Neuro-lymphomatosis" believing that the primary changes involved the nervous system only, but experience has shown that certain tumours commonly found involving the ovary, liver, kidneys, lungs, Bursa of Fabricus, muscles, etc. are also manifestations of the characteristic lymphoid infiltrations. Therefore, the accepted use by scientists now, is for the broader and more satisfactory term Lymphomatosis - the lay poultry farmer still, of course, continues to refer to the disease as "paralysis." Indeed, this has often led to a good deal of confusion, because fowls frequently suffer from paralysis, the causes of which are numerous, and which often vary from outbreak to outbreak.

Attempts to transmit the disease experimentally by injection have not been very satisfactory - in the majority of instances, less than 25% successful transmissions being recorded.

In a number of cases, where greater success has been achieved, the control birds also suffered, therefore such experiments were nullified. The question of obtaining adequate controls has always proved a difficult one, owing to the apparent widespread nature of the disease in latent form.

One of the striking features of outbreaks from the clinical aspect has been the fact that in many cases the disease appeared to have an hereditary aspect, being related to certain "strains" of birds. Warrack and Dalling (1932) concluded that the male bird might play a part in transmission, although subsequent papers by these and other conscientious workers showed that the primary cause - not yet proved to be a virus - frequently appeared to be inherited from the dam. In this connection, a number of hens with characteristic eye lesions breed chicks, which, under certain circumstances, develop the disease proper in its typical form. Therefore, the culling of breeding stock for birds so affected is an essential part of the routine adopted by farmers where measures are being taken for the eradication of fowl paralysis on disease ridden farms.

Recently, experiments have shown that fowl paralysis may be infectious. In this respect, the results of Fraser and Johnson (1938) at the Rowett Research Institute are outstanding. They concluded, following several years work using Greenwoods in-bred brown leghorn stock, that an infective factor was the primary cause of the disease and therefore that it was infectious

in the true sense of the word.

In spite of negative transmission experiments in 1921, Kaupp considered the disease infectious, an opinion later shared by Doyle (1926) and Johnson (1932). However, this is quite contrary to the experience of many veterinary surgeons in this country, the writer among them, who have encountered a number of outbreaks of fowl paralysis in which the disease had apparently localised itself to one strain or breed of poultry, whereas another strain of birds reared together and in contact with the affected ones remained free. Similarly, at Laying Tests there is ample evidence to show that only one bird in a pen of twelve will suffer from fowl paralysis, the remainder showing no external signs of the disease.

Emmel (1937) has approached the problem from a somewhat different standpoint, and considers that he can reproduce fowl paralysis or leukaemia by injecting certain Salmonella organisms intravenously into susceptible young chicks. Patterson (1934) also concludes that neurolymphomatosis gallinarum, lymphoma, erythro- and myeloid-leucosis are all expressions of the same disease, capable of spreading by direct and indirect pen contact and also by rearing chicks on infected litter. Lee and his colleagues (1937) reach the same conclusions and believe that the transmissible agent is a filtrable virus.

Dalling (1938) repeated Emmel's experiments, using bacterial cultures sent over by him from America, but "was entirely unable to set up any resemblance to fowl paralysis by their use in parasitized or non-parasitized birds." Dalling did not, however, do any haematological work similar to that by Emmel on Haemocytoblastosis.

Gray (1938) considers that micro-organisms (Streptococcus viridans and Staphylococcus aureas) which he has cultivated from the fluid expressed from enlarged peripheral nerves in typical cases of fowl paralysis may be related to the primary causation of the disease, but it is not improbable that these germs are post-mortem invaders, because the present writer has failed to find them in any fresh specimens examined.

Finally, it should be mentioned that nearly all workers have noticed the large numbers of intestinal parasites which complicate the fowl paralysis disease picture. One of the reasons why parasites have been so intimately associated with fowl paralysis is because they may in themselves be a cause of "paralysis" in poultry. The position is so closely linked up, many investigators are still uncertain whether the intestinal paralysis found in a number of affected birds (i.e. in fowl paralysis) allows the parasites to get a foothold and so establish themselves, or whether the parasites are a necessary predisposing cause for the onset and development of fowl paralysis proper.

Numbers of investigators have associated heavy infestations of coccidia, davainea, or heterakis with typical cases of fowl paralysis, but others, notably Hamilton and Blount (1932), Biely, Palmer and Lloyd (1933) and Warrack and Dalling (1933) have shown that there is apparently no direct relationship between the post-mortem findings regarding intestinal parasites and fowl paralysis. The latter workers showed clearly that the disease could develop in young birds specially reared in parasite-free surroundings. Nevertheless, in true fowl paralysis (naturally contracted) the high percentage of cases associated with parasites of the intestines appears as a striking clinical feature.

Those who have transmitted the disease successfully by injection are agreed that the incubation period of fowl paralysis is long - probably not less than 6-8 weeks. This fact alone may, therefore, nullify the conclusions drawn by some investigators as to the relationship between parasites and true fowl paralysis, because at the time of the death of the bird the parasites which were present at the onset of the disease may have disappeared, to leave little or no trace of their activities discernible by the naked eye.

THE LITERATURE CONCERNING THE BLOOD PICTURE IN CASES OF FOWL PARALYSIS

Owing to the very extensive number of outbreaks which occurred during 1931-32 in England, a number of laboratory workers endeavoured to find a method for the diagnosis of latent fowl

paralysis. Seagar (1933) published a rather startling paper in which he claimed to be able to diagnose the disease - even in its latent stages - by examinations of blood films. Seagar formed the opinion that a polynuclear increase occurred during the late incubation and early acute stages of the paralysis, but that during the more advanced manifestations of the disease, a lymphocyte reaction followed. This formed the basis of the so-called Cyto-diagnosis test which was used commercially in this country for a number of years.

The following are Seagar's figures for healthy poultry:-

Poly-morphs	Eosino-philes	Basi-philes	Total lymphocytes	Monocytes
34	5	2	54	5

With reference to data on normal blood samples, the writer, in connection with his Royal College of Veterinary Surgeons Fellowship thesis, examined 58 fowls taken from flocks considered in good health. However, from a study of their total red and white cell counts and post-mortem findings, only 20 of the birds were considered to be quite healthy. The percentage variation between individual birds even then was considerable, as can be seen from the following:-

N.	E.	B.	L.L.	S.L.	T.L.	Mono.
20.3	3.0	2.4	11.7	62.2	73.9	.4
(11-36)	(.5-7.3)	(.7-5.3)	(7.7-23.5)	(43-73)	-	(0-2)

Seagar stated that in the early stages of fowl paralysis a polynuclear increase from 39% (poly and eo combined) to 47% occurred with a concomitant fall in the lymphocytes from 54% to 48%. Since this formed the basis of the "Cyto-diagnosis test" it was clear that Seagar considered the alteration to be specific, whereas the reverse is in fact the case. For example, here is the differential count of (1) three typical B.W.D. reactors, and (2) four pullets suffering from parasitic anaemia.

		P.	E.	B.	L.	M.
1.	B.W.D.	42.3	1	3	53.3	.3
2.	Parasitic anaemia	45.2	.6	3.4	50.4	.4

According to Seagar's statement, all these birds would be classed as early fowl paralysis, yet they were in fact suffering from two entirely different diseases.

Succeeding the "poly-phase" Seagar records a lymphocytic reaction in which the non-granular leucocytes rise to approximately 70%, whilst the granulocytes fall to 30%. Presumably, therefore, a number of the normal birds quoted above (Blount 1931) were suffering from a fairly advanced stage of lymphomatosis.

Further, it should be noted in order to effect the above changes described by Seagar, the differential count would presumably have to pass through the normal range, i.e. from the polynuclear to the lymphatic phase. Therefore, it would be impossible to differentiate between those birds whose blood was

actually normal and others who were really in a transition stage of fowl paralysis yet still showing a normal blood picture.

Blakemore (1934) also confirmed the uselessness of Seagar's method of diagnosis and decided that "a slightly increased total leucocyte count may be present in cases of fowl paralysis. This rise, however, does not appear to be a consistent feature of the disease, and the fluctuations in the numbers of leucocytes present in normal fowl blood are in themselves sufficient to make a total leucocyte count valueless for diagnostic purposes. In view of the non-specific variations which occur in the polynuclear cells, it also appears extremely doubtful if any significance can be attributed to the differential percentage leucocyte counts in cases of fowl paralysis, without reference to the total leucocyte count."

In the second part of his paper, Blakemore (1934) considers the features of the differential count in fowl paralysis, but unfortunately this portion of his work appears open to several important criticisms:-

(1) The cells which he grouped together as "polymorphs" included both neutrophiles, eosinophiles and also basiphiles. There seems absolutely no justification for this procedure, since the function of these cells is not in any way comparable, and neither does the circulating basiphile have a polymorphic nucleus.

(2) No attempt is made to distinguish young from old lymphocytes, therefore, Blakemore fails to show whether the

lymphoid system is, or is not, reacting to a stimulus. Clearly a most important point to have neglected, and of great importance in any haematological study of fowl paralysis.

(3) Although the diseased birds were subjected to post-mortem examinations so that fowl paralysis could be diagnosed by histological examinations of diseased nerves, no data was presented to show the degree, if any, of intestinal parasitosis accompanying the neuro-lymphomatosis. This is essential in any study of the differential count of diseased poultry.

(4) Only total red cell counts were carried out with reference to erythrocytes - there was therefore no reference to any question of erythropoietic stimulation (erythroblastosis) as a feature in fowl paralysis.

(5) No indication was given as to the extent of the neural lesions in the 18 diseased fowls examined, i.e. slight, marked, or gross enlargements.

(6) Concerning the four groups of normal fowls, no data was given as to their breed, age or sex.

(7) His conclusions were not on a comparative basis, for he failed to study the blood of poultry suffering from diseases other than fowl paralysis.

Dealing with the blood picture in fowl paralysis, Furth (1934) found that the number of lymphocytes in the blood was occasionally increased. The highest leucocyte count he observed

was 140,000/c.mm., of which 92% were lymphocytes mostly of small size. Bayon (1931) considered the leucocyte count high in fowl paralysis due to an increase of lymphocytes. Jungherr (1934) also considered that suspicious and positive birds seemed to show a rise in the leucocyte count, whilst Emmel (1935) has stated that although the white cells may vary from as wide a range as 40,000 - 160,000/c.mm., in the majority of cases they are between 45,000 and 75,000/c.mm. Among a second group of birds (40 in number) suffering from lymphomatosis and varying in age from 14 to 98 weeks of age, Emmel found the total leucocyte count to vary between 43,000 and 83,000/c.mm.

The essential feature of Emmel's (1936) study of the blood in fowl paralysis was his demonstration of the process of haemocytoblastosis, which he considers an essential factor in the development of this disease. In describing this condition, Emmel writes "in addition to immature forms of blood cells of any type, a variable number of degenerative forms of mature and immature blood cells is to be found in the peripheral circulation. Haemocytoblastosis is also characterised by a variable increase in the total number of white blood cells ..... the erythrocyte count in active haemocytoblastosis is usually, at least, slightly under normal."

Although the course of this blood abnormality is variable, Emmel expects to find all birds affected with fowl

paralysis showing it in some degree. His principal findings were (1) a varying number of degenerative cells of all types, principally eosinophiles, polymorphonuclears and lymphocytes, (2) Immature lymphocytes in over 70% of cases, together with a notable increase in the number of vacuolar lymphocytes, and (3) Premyelocytes, and polychrome erythrocytes or basiphilic erythroblasts.

Briefly, therefore, Emmel considers that "in the development of fowl paralysis, haemocytoblastosis is as essential a factor as the potency of the endotoxin of the causal micro-organism. (Salmonella)." These bacteria are believed by him to gain access to the blood-stream through the intestinal mucosa becoming injured due to intestinal parasites - there to initiate the haemocytoblastosis process.

Finally, it is to be remembered that although Emmel considers haemocytoblastosis an essential process in the development of many pathological manifestations, including leukaemia, he does not claim that this blood disorder in itself will necessarily lead to fowl paralysis, for other factors require to be co-related with haemocytoblastosis before fowl paralysis results.

(Emmel further states that during the early period of his investigations, he observed many outbreaks of fowl paralysis which also revealed a high incidence of leukaemia.

However, this is contrary to experience in this country where outbreaks of leukaemia are rare, and fowl paralysis is extremely common. Odd cases of leukaemia are encountered at all times of the year, but it cannot be said that there appears to be any real foundation, from clinical experience gained in the field, for attempting to co-relate the two diseases aetiologically).

PERSONAL OBSERVATIONS REGARDING THE BLOOD PICTURE IN FOWL PARALYSIS

From the nature of the disease process concerned in fowl paralysis, namely, an infiltration of parts by lymphoid cells, it is clear that it would be unreasonable to expect the blood picture to be constant. However, this does not necessarily mean that there are no important or diagnostic features to be learnt from a study of the blood cells.

For practical purposes, three clinical types can be recognised:-

(1) Typical cases characterised by paralysis involving the leg or wing.

(2) Birds whose only ante-mortem signs are blindness (due to progressive iritis), semi-emaciation and quiescence of the ovary, and

(3) Those examples of the disease known as visceral lymphomatosis in which lymphomata are disclosed post-mortem, involving some major organ, e.g. kidneys, lungs, ovary, liver, etc.

Each type may be complicated by the presence of numerous internal parasites, with or without associated naked eye lesions. Therefore, in an examination of birds taken from a flock where fowl paralysis is prevalent, it would be common to find birds as follows:-

(a) Fowl paralysis - right sciatic and right brachial plexus grossly enlarged.

(b) Fowl paralysis - fixation of both pupils, complete iris depigmentation, extensive duodenal coccidiosis.

(c) Fowl paralysis - lymphomata of both kidneys.

(d) Fowl paralysis - impaction of the gizzard, cranial mesenteric nerve enlarged, numerous davainea in the duodenum - also ascaridia. Ovarian tumour (lymphoma).

An attempt has not been made to group all cases of the disease together, believing that there is a common blood picture for all, but rather to consider them according to their clinical features - that is, whether the diagnosis of fowl paralysis was dependant upon nerve enlargements, lymphomatic tumours, or a characteristic iritis. At the same time, the question of parasites has been taken into account, together with the extent of their associated lesions. (It should be pointed out that all the birds suffering from fowl paralysis were natural cases of the disease - none being of an experimental type.)

The following analysis of twelve cases of fowl paralysis with typical nerve lesions shows:-

	P.	E.	B.	L.L.	S.L.	T.L.	Mono.	W.b.c's.
Fowl Paralysis	31.4	2.9	3.74	15	46.6	61.6	.44	34,000
Normal birds	20.3	3.0	2.4	11.7	62.2	73.9	.4	27,000

The leucocytosis is slight but definite, and is neutrophilic in type, with a corresponding depression of the lymphocytes. It should be noted, however, that the ratio between large and small lymphocytes in these cases of true fowl paralysis is approximately 1:3, whereas in normal birds this is nearly doubled. There appears therefore to be an actual increase in the number of circulating large lymphocytes, even though the small variety is reduced. Presumably a stimulus to the lymphoid system is in operation, but the blood-stream would appear to be unfavourable for the normal retention and maturation of the cells so produced. This is further shown in numbers of the medium sized lymphocytes by the appearance of vacuoles, or by a clumping or budding of the cytoplasm - a feature also observed in the smallest lymphocytes.

The differential count shows, in about 75% of cases, a reversal of the normal eosinophile-basiphile ratio; for, contrary to expectations based on studies of bloods of numerous mammalian species in health and disease, the basiphile in fowl paralysis predominates. This fact seems to have escaped the notice of Blakemore, Seagar and others, yet its predominance may be suggestive of, though not specific for, a diagnosis of

neuro-lymphomatosis. Further, it should be noted that numbers of the basiphiles are of the earliest types recognisable, in which the stroma of the cytoplasm can be seen clearly owing to the lack of actual basic "granules." This fact, together with the character of the single rounded nucleus, suggests that the bone marrow is being stimulated to produce and release (before having acquired their full complement of granules) numbers of immature basiphile myelocytes.

In about 40% of cases, typical panchromatic premyelocytes are also observed, though these never become numerous or as characteristic as in caecal coccidiosis.

Further evidence of the unfavourable state of the blood-stream in fowl paralysis towards circulating blood cells is seen in an examination of the red cells. Whilst primary erythroblasts are never encountered, there is clear evidence of both regeneration and degeneration in the erythrocyte picture. Anisocytosis and polychromasia are common, as is plastid formation and pyknosis of the red cell nucleus, many of which show "notches." The majority of the normocytes are fully matured, but the presence of nuclear remnants and some early erythrocytes together with the differential count confirms Emmel's suggestion that haemocytoblastosis may be a factor associated with the aetiology of fowl paralysis.

Therefore, although a casual glance at the fowl paralysis (nerve type) blood cell picture on paper in terms of

percentages and numbers shows little to be considered of importance, an examination of the actual film under the microscope is much more instructive for it presents:-

(1) Few of the advanced features of anaemia, often noted in coccidiosis.

(2) Fewer eosinophiles and more basiphiles than normal.

(3) A decrease in the number of small lymphocytes - associated with degenerative features such as vacuolation and "budding."

(4) Four leucocytes per field instead of the normal three.

These features are not necessarily specific, nor do they occur in every case, but when they, alone, are noted they are strong presumptive evidence of fowl paralysis.

Although these statements are true for typical cases of neuro-lymphomatosis-gallinarum, a careful analysis of the data obtained from an examination of 10,000 leucocytes - in conjunction with the post-mortem findings of the 22 animals concerned - shows that additional conclusions are justifiable. Thus:-

(1) There is some evidence to indicate that in uncomplicated cases of fowl paralysis, the greater the degree of nerve enlargement so there is a tendency for the blood picture to become more neutrophilic, but the LL:SL ratio remains narrow.

Therefore, as the disease progresses the polymorphs will tend to increase at the expense of the small lymphocytes. This is absolutely contrary to Seagar's statement that a marked rise in the lymphocyte count occurs in advanced stages of the disease.

(2) Similarly, the total leucocyte count will be seen to fall with an advancement of the disease process, and therefore birds with gross enlargements of the affected nerves usually show lower WBC counts compared with those where the nerve lesions are slight. Thus:-

Ref:	P.	S.L.	T.L.	WBC	Degree of fowl paralysis	Parasites
201	24.7	48.3	68.3	28,000	+	-
298	31.7	35.3	61.0	19,500	+++	+
294	39.7	20.7	53.7	14,000	++++	-
116A	20	50.3	65	36,000	Iritis ++++	-
116B	26.3	25.3	52.6	27,200	Iritis ++++ after 21 days	-
116C	40.3	11.6	29	18,500	Iritis ++++ after 33 days	-
146	50.7	24	38.3	28,000	Lymphoma ++	Mixed ++
225	85.3	8.3	13.6	68,000	Lymphoma ++++	-

(3) In the case of birds suffering from actual lymphomata of the viscera - contrasted with those exhibiting nerve lesions only - the larger the tumour concerned the greater

the percentage of neutrophiles in the differential blood count. Blakemore (1934) also found that birds with tumours and enlarged nerves had total leucocyte counts greater than in the cases with nerve enlargements alone, or where no macroscopic lesions were found. Thus:-

Fowl Paralysis Cases - Blakemore

A	B	C	D
33,600 Tumours and enlarged nerves.	31,800 Enlarged nerves	29,700 No macroscopic lesions.	25,100 Normal fowls.

(4) Although uncomplicated fowl paralysis appears to cause a neutrophilia with slight though obvious depression of the total WBC count, if the case is one complicated by coccisiosis, then a depression of the neutrophiles and basiphiles occurs. This is particularly well seen in the following two cases of bi-lateral iritis, of fowl paralysis origin, in which massive infestations of duodenal coccidia were found post-mortem:-

Ref:	P.	E.	B.	M.L.	S.L.	T.L.	Date
121A	24.7	1.7	1.3	19.6	45.7	72	20/7/38
121B	9	.3	.7	8	78.3	90	18/8/38
276A	17	5	3	52	6	75	30/8/38
276B	15	3	2.3	8.7	62	79.4	8/9/38

The influence of coccidia on the lymphoid system of the fowl paralysis hen would seem to be one of stimulation. This is particularly well seen in bird reference 276A in which

the result of the stimulus can be seen to be affecting the medium sized lymphocytes, whereas nine days later, this is reflected in a marked rise in the percentage of small lymphocytes. These latter cells are therefore usually predominant, and in addition there is often no excess of basiphiles over eosinophiles.

There seems little doubt that this is the probable explanation for Seagar's assumption that a lymphocytosis accompanies the later stages of fowl paralysis. He was, in fact, recording the blood picture of neuro-lymphomatosis-gallinarum, complicated by advanced or chronic coccidiosis. His belief that the early acute stages of the disease are characterised by a polynuclear increase appears to be correct; but, as indicated above, it is also probable that in uncomplicated cases the neutrophilia increases according to the degree of lymphoid infiltration.

(5) Moderate infestations of caecal worms (Heterakis) do not appear to disturb the average blood picture in fowl paralysis greatly, although the fall in the neutrophiles is one which is absolute as well as relative, but this is counteracted by the greater retention of the blood-stream for small lymphocytes. The preponderance of basiphiles over eosinophiles is not disturbed, nor are the monocytes. Thus:-

	P.	E.	B.	L.L.	S.L.	T.L.	M.	WBC's
Average fowl paralysis count	31.4	2.9	3.74	15	46.6	61.6	.44	34,000
Fowl paralysis and heterakis infestation	23.4	2.0	3.5	15.65	55.35	71	.45	38,000
Normal fowls	20.3	3.0	2.4	11.7	62.2	73.9	.4	27,000

The following example will show that the blood picture, if interpreted correctly, can be of great assistance in the diagnosis of fowl paralysis - even when the clinical findings are doubtful.

Subject - White Leghorn No: 262

Observed limping slightly (18/10/37), an examination of the affected limb gave no definite clue as to fowl paralysis. A differential count showed:-

P.	E.	B.	L.L.	S.L.	LL:SL	M.
22	3.3	5	5.3	63	1:12	1.3

The majority of the basiphiles were early myelocytes, and although most of the erythrocytes were mature, many showed notches and others pyknosis. These facts together with the reversed normal eosinophile-basiphile ratio were suspicious of true fowl paralysis, but the LL:SL ratio was wide which is not found in neural types of the disease.

Nearly three weeks later (4/11/37), during which time the paralysis had steadily increased, the blood picture showed:-

P.	E.	B.	L.L.	S.L.	T.L.	M.
14.7	2.7	1.6	6	75	81	0

The steady increase in small lymphocytes and decrease in neutrophiles was obvious, whilst the basiphile-eosinophile ratio was reversed and the SL:LL ratio still wide. This was therefore not consistent with a diagnosis of fowl paralysis, but

typical of progressive complicated duodenal coccidiosis. Post-mortem examination revealed a star fracture of the femur, duodenal coccidiosis and an infestation of caecal worms. There were no signs of true fowl paralysis. The fracture may have accounted for the pre-myelocytes present.

COCCIDIOSIS

Clinical Notes

Coccidiosis is probably the most important disease of poultry, because it attacks birds of all ages causing greater loss even than fowl paralysis. There are two main types of coccidiosis - caecal and duodenal - although a third less common form involving the jejunum is equally important on account of its associated high mortality.

The causal organism - a non-flagellate protozoan parasite - attacks the cells lining the intestines, and in the majority of instances the pathological changes appear to be limited to the gut, but in some cases the post-mortem findings also reveal feathery, degenerate areas in the liver.

Excreted in the faeces as a resistant oocyst, the avian coccidium becomes infective only after exposure for about 48 hours to the atmosphere, with suitable temperature and moisture. At this stage, therefore, a poultry keeper can break the coccidiosis life cycle by cleaning out the poultry houses every 24-36 hours, or by moving the birds in "folding-unit" houses on to fresh ground every day.

Following the swallowing of the infective oocyst, numerous microscopic merozoites form, to cause destruction of the intestinal epithelium. Later, the parasite undergoes a change necessitating the formation of different "sexes." In a

typical case of duodenal coccidiosis, the large round female macrogametes can be seen developing in the columnar epithelial cells - resting between the central nucleus and the free ciliated border. Finally, typical oocysts can be seen free in the duodenal contents, many with a micropyle, others sealed and therefore presumably fertilised by micro-gametes which represent the male element in the gametogony phase. The oocysts of duodenal coccidiosis often differ in size from those of the jejunal and caecal species, but it is usual in practice to find "mixed" infestations.

Caecal coccidiosis commonly attacks chicks aged 3-10 weeks, and is first detected in per-acute cases by the sudden appearance of dysentery. Affected chicks appear drunken, ruffled in the feather and usually die after an illness lasting only one or two days; post-mortem examination showing the caecal tubes full of blood containing myriads of oocysts.

Duodenal coccidiosis is quite different in many respects. Affected birds are older, usually aged about 3-8 months; they seldom die rapidly, but become emaciated, anaemic and lethargic. Post-mortem examinations show few specific features, but catarrhal or patechial lesions in the first part of the small intestines are common.

In coccidiosis caused by Eimeria necatrix there are well marked oedematous and inflammatory lesions involving the jejunum, and the symptoms differ from those of the duodenal type

because of the higher mortality and absence of emaciation.

Following acute outbreaks of coccidiosis, there are always large numbers of survivors that suffer from its after-effects for many weeks, and in chronic caecal coccidiosis, it is common to find large solid cheesy masses involving the blind guts. (Pus in the hen is solid, believed to be due to an absence of proteolytic ferment (leuco-protease) in the neutrophiles).

THE BLOOD PICTURE IN AVIAN COCCIDIOSIS

The literature dealing with the blood picture in coccidiosis is sparse; indeed, except for isolated references to anaemia little is to be found on the subject.

Caecal Coccidiosis

At first sight, the data obtained from an examination of the blood of fowls suffering from caecal coccidiosis (Table 17) appears quite incapable of analysis. There seems little co-relation between the disease process and the wide deviations from normal which occur in all groups of blood cells, for these range from 2.5 to 70 polymorphs, 0 to 12 eosinophiles, 0 to 6 basiphiles, 27.8 to 92 lymphocytes and 0 to 38 monocytes.

However, the 20 cases examined readily group themselves according to the age of the affected birds - (a) 4-5 weeks, (b) 6-7 weeks and (c) 10-12 weeks; and, by also carefully considering the post-mortem findings of each bird, it has been found possible to piece parts of the story together.

In the case of fowl paralysis, no standard blood picture is obtainable, because the disease has many different pathological manifestations apart from the question of accompanying parasitic infestations; but, in caecal coccidiosis, which is a disease entity in itself, it was hoped that there would be at least a constant differential count, even if not the blood picture generally. A little consideration, however, will show that this is a vain hope, for not only can the infestations be heavy or light, with the affected birds either severely ill or only just "off-colour," but, in chronic cases, extensive pus formation may also be present. Additional complications are to be anticipated in per-acute cases which are characterised by severe internal (intestinal) haemorrhage, and also in the post-coccidial cases of haemocytoblastosis.

In general, the average blood picture in caecal coccidiosis shows a marked neutrophilia (both relative and absolute), an increase in the basiphiles, large lymphocytes and monocytes and considerable reduction in the small lymphocytes. The total leucocyte count is rather lower than normal, as is the percentage of haemoglobin. Owing to the close association between coccidiosis and fowl paralysis it is interesting to contrast the two blood pictures, thus:-

	Fowl paralysis	Caecal coccidiosis
Polymorphs	31.4	35.28
Eosinophiles	2.9	2.53
Basiphiles	3.74	3.34
Large lymphocytes	15	19.71
Small lymphocytes	46.4	35.81
Total lymphocytes	61.6	55.49
Monocytes	.44	3.23
White blood cells	34,000	23,800

The general appearance of the caecal coccidiosis blood picture resembles that of the average case of fowl paralysis, especially if the excess of basiphiles over eosinophiles is considered of importance. However, as can be seen above, there is a marked reduction in the smallest lymphocytes, so that the ratio LL:SL is thus lowered as 1:2. Little attention should be paid to the relative monocytosis recorded, for this is largely due to the inclusion in Table (1) of one bird (Reference 284) in whose differential count there were 38% of monocytes. If this case is excluded, the figure above (3.23%) will then read 1.4%.

CAECAL COCCIDIOSIS - Chicks aged 4-5 weeks.

Chicks of this age are commonly affected with caecal coccidiosis in all its forms. The blood picture differs little from that of the average coccidiosis case:-

	Caecal coccidiosis - all cases	Caecal coccidiosis - aged 4-5 weeks	Normal
Polymorphs	35.28	33.6	20.3
Eosinophiles	2.53	1.9	3
Basiphiles	3.34	3.8	2.4
Large lymphocytes	19.71	19.55	11.7
Small lymphocytes	35.81	38.55	62.2
Total lymphocytes	55.49	58.10	73.9
Monocytes	3.23	2.1	.4
White blood cells	23,800	18,570	27,000

Contrasted with the normal differential count in fowls, it will be seen that there is a further depression of eosinophiles, making the basiphiles twice as numerous. Also, in comparison with the average case of coccidiosis, the total leucocyte count is lowered until it is a good deal below that of a healthy fowl.

Perhaps the most striking feature of the blood of these young affected chicks is the widespread occurrence of haemycytoblastosis - a feature not disclosed by the differential count. This is discussed later. Chicks dying from caecal coccidiosis do not always show the severe leucopenia (5,000 WBC/c.mm.) present in bird 196 (Table 17), but there is often a complete depression of the eosinophiles. It is interesting to note that the chick (No: 258) with the lowest percentage of haemoglobin (58%) was not one suffering from internal haemorrhage - nor did it show many clinical signs of the disease.

There appears to be a superficial relationship between the approximate number of coccidiosis parasites present and the neutrophilia, and this latter condition is also expected to be marked where pustular typhlitis is observed. Thus:-

Degree of infestation	% Neutrophiles
++	28
+++	31
++++	34.4
+++ pus +	43.7

One bird, No: 210, however, refutes this suggestion for the percentage of neutrophiles in this case was only 2.5%, yet pus was present in the caecal tubes in addition to a heavy infestation of coccidia.

CAECAL COCCIDIOSIS - Chicks aged 6-7 weeks.

Three sub-acute cases of the disease showed a well marked neutrophilia, but little evidence was obtained to show whether this was solely due to the stimulation afforded by coccidial "toxins," or whether at this stage, pathogenic bacteria were also operative. In these three cases, the LL:SL ratio was lowered still further to 1:1.76 - the total leucocyte count having risen to normal (28,300 cells/c.mm.).

Chicks aged 6-7 weeks suffering from chronic caecal coccidiosis show marked blood changes. There is a leucopenia, mainly of the neutrophiles, depression of the eosinophiles and

basiphiles, and a relative lymphocytosis. The total leucocyte count is about two-thirds that of the normal fowl, yet the percentage of lymphocytes is double that of chicks the same age but suffering from sub-acute caecal coccidiosis. Thus:-

Caecal coccidiosis - chicks aged 6-7 weeks

	Sub-acute	Chronic	Normal
Polymorphs	57.3	15.1	20.3
Eosinophiles	1.72	1.42	3.0
Basiphiles	3.05	2.08	2.4
Large lymphocytes	13.61	31.29	11.7
Small lymphocytes	23.89	47.49	62.2
Total lymphocytes	37.5	78.8	73.9
Monocytes	.5	1.68	.4
White blood cells	28,300	19,300	27,000

Chicks suffering from chronic coccidiosis sometimes are a prey to their more vigorous pen-mates, and therefore may suffer from the effects of cannibalism, e.g. Bird 284. This bird was removed from a pen where numbers of chicks had suffered from coccidiosis for several weeks previously. It had become disembowelled through cannibalism, but was still alive when the blood sample was taken - the post-mortem examination did not disclose any apparent cause for the monocytosis. Another bird (No: 140) taken from this same pen looked perfectly healthy and showed a similar blood count (excluding the monocytosis) but it

was complicated by marked haemocytoblastosis - the haemoglobin percentage and total leucocyte count were normal. Thus:-

	Ref: 284	Ref: 140
Polymorphs	16	16.5
Eosinophiles	0	2.25
Basiphiles	6	4.25
Lymphocytes	40	76.75
Monocytes	38	.25
White blood cells	2,000	26,800
Haemoglobin	48	75

CAECAL COCCIDIOSIS - pullets aged 10-12 weeks.

During the carrying out of certain feeding experiments at the School of Agriculture, Plumpton, on Table Sussex poultry, outbreaks of coccidiosis interrupted the work, and five typical sub-acute cases showed the following blood counts:-

	Sub-acute coccidiosis	Sub-acute coccidiosis chicks aged 6-7 weeks	Normal
Polymorphs	43.8	57.3	20.3
Eosinophiles	5.4	1.72	3
Basiphiles	2.7	3.05	2.4
Large lymphocytes	19.9	13.61	11.7
Small lymphocytes	27.2	23.89	62.2
Total lymphocytes	47.1	37.5	73.9
Monocytes	1.04	.5	.4
Haemoglobin	58	61	74
White blood cells	41,600	28,300	27,000

It will be seen that the reaction is, in the main, similar to that in younger chicks suffering from the same disease. However, although the polymorph percentage of the differential count is lower than that of the younger chicks, the total leucocyte count is considerably higher. At the same time, there is a marked stimulation of the eosinophiles and large lymphocytes - the LL:SL ratio remaining narrow. This greater response on the part of the older pullets is probably due to their age and freedom from infestations during their earlier days.

DUODENAL COCCIDIOSIS

A series of 15 cases of duodenal coccidiosis in growing stock have been studied. They show some interesting results particularly in conjunction with those obtained from chicks suffering from caecal coccidiosis.

All the birds were aged 3-5 months and none of the cases were per-acute, but unfortunately the majority were complicated by other parasites notably caecal coccidia, davainea, heterakis and ascaridia. The differential counts were remarkably steady, except in the case of bird 282 which was in the most advanced stages of the disease and about to die.

The blood picture in general is very similar to that shown by pullets aged 10-12 weeks suffering from sub-acute caecal coccidiosis, thus:-

	Duodenal coccidiosis	Sub-acute caecal coccidiosis	Normal
Haemoglobin	63.1	58	74
White blood cells	32,900	41,600	27,000
Polymorphs	41.2	43.8	20.3
Eosinophiles	4.2	5.4	3
Basiphiles	2.8	2.7	2.4
Large lymphocytes	19.7	19.9	11.7
Small lymphocytes	31.4	27.2	62.2
Total lymphocytes	51.1	47.1	73.9
Monocytes	.7	1.04	.4
No: of cases	12	5	20

In duodenal coccidiosis there is a definite marked neutrophilia and a slight eosinophilia, as a consequence contrasted with the normal blood picture there is a 50% reduction in the number of small lymphocytes. Although neither the leucocytosis nor the percentage of neutrophiles is as high as in sub-acute caecal coccidiosis, it is a marked feature of the blood picture. Similarly, the increase in large lymphocytes is characteristic, so that the normal LL:SL ratio of 1:5.3 drops to 1:1.6 - the monocytes showing no change.

Whereas in young chicks aged 6-7 weeks suffering from sub-acute caecal coccidiosis the basiphiles were dominant over the eosinophiles, the position is reversed in older birds

(aged 10-20 weeks) no matter whether affected with caecal or duodenal coccidiosis. If the infestation is of recent origin the eosinophiles are depressed, but the percentage appears to rise the more the disease becomes chronic. Therefore, if birds are found suffering from duodenal coccidiosis complicated by other internal parasites, the eosinophile percentage is accordingly higher than in uncomplicated duodenal coccidiosis. Thus:-

	Eosinophiles	Basiphiles
Caecal coccidiosis - birds aged 4-5 weeks	1.9	3.8
Duodenal coccidiosis - birds aged 12-20 weeks	4.2	2.8
Sub-acute caecal coccidiosis - birds aged 10-12 weeks	5.4	2.7
Duodenal and caecal coccidiosis, etc. birds aged 12-20 weeks	7.7	2.6

This suggestion cannot be said to apply to all cases, but it is interesting to consider the possibility that the eosinophilia encountered in coccidiosis may be a cell response to continued intestinal irritation. (See discussion on Eosinophilia - page 174).

One bird (No: 282) examined whilst dying showed 70% of neutrophiles including 10% of premyelocytes and in this instance there was a total suppression of the eosinophiles, basiphiles, large lymphocytes and monocytes, and a haemoglobin percentage of only 35% associated with marked features of haemocytoblastosis.

Two other atypical cases of duodenal coccidiosis were:-

(1) Light Sussex Cock No: 286, aged 12 weeks, suffering from a mixed intestinal parasitosis - coccidia of both types, capillaria, and davainea being present.

(2) Light Sussex pullet No: 135, dying from duodenal coccidiosis, together with advanced amyloid degeneration of both kidneys.

In each of these cases, the neutrophiles were reduced to 9.7 and 14% respectively, and although both had a high percentage of large lymphocytes, the pullet's most outstanding feature was a basiphilia of 34%.

DUODENAL COCCIDIOSIS - Blood Data

Reference	282	170	293	145
Degree of Coccidiosis	+++	++++	++	+++
Haemoglobin	35%	64%	85%	55%
White blood cells	34,000	35,800	31,200	30,000
Polymorphs	70	49.3	38.7	48
Eosinophiles	0	2.3	0	2.3
Basiphiles	0	2.3	2.7	1.0
Large lymphocytes	2	16.3	6	32.7
Medium lymphocytes	20	9.7	9	6.3
Small lymphocytes	8	19.7	41.6	9.3
Total lymphocytes	30	45.7	56.6	48.3
Monocytes	0	.4	2	.4
Remarks	Dying	Ill	Caecal cocc. ++	Caecal cocc. ++

DUODENAL COCCIDIOSIS - Blood data

Reference	194	283	199	Ticehurst
Degree of Coccidiosis	++	++	++	+
Haemoglobin	50%	45%	78%	72%
White blood cells	30,000	45,000	38,800	-
Polymorphs	49.3	47	39.7	39
Eosinophiles	12	9.7	6.3	3
Basiphiles	.7	4.7	1.3	5
Large lymphocytes	23	16.7	30	11
Medium lymphocytes	3	11.3	15	4.5
Small lymphocytes	11.3	10.3	8.4	36.5
Total lymphocytes	37.3	38.3	53.4	52.0
Monocytes	.7	.3	0	1.0
Remarks	Mixed infestation.	Mixed infestation.	Ascaridia ++	Duodenitis. Heterakis ++

DUODENAL COCCIDIOSIS - Blood data

Reference	A.H.	H.C.	T.H.	212	Average (12 cases)
Degree of coccidiosis	+++	++	+++	++++	-
Haemoglobin	70%	70%	-	70%	63.1%
White blood cells	-	-	-	18,500	32,900
Polymorphs	30	36.3	23.8	23.8	41.2
Eosinophiles	2	1	3.1	8.5	4.2
Basiphiles	3	4.7	2.8	5.5	2.8
Large lymphocytes	9.5	13.7	2	14	14.8
Medium lymphocytes	9.0	4.6	14.3	10.8	9.8
Small lymphocytes	45.0	38.7	54	34.7	26.5
Total lymphocytes	63.5	57.0	70.4	59.5	51.1
Monocytes	1.5	1.0	0	2.75	0.7
Remarks	Caecal ++	Heter-akis ++	Ascar-idia +++	Duodenitis ++++ Davainea +	

HAEMOCYTOBLASTOSIS

Literature

A survey of the literature on fowl paralysis in this country fails to show one paper dealing with haemocytoblastosis, and therefore as far as can be ascertained this is the first attempt to confirm Emmel's work in the British Isles.

Johnson (1934) was the first to describe it in connection with the development of fowl paralysis or leukaemia, and the term was then used to designate the presence of haemocytoblasts in the peripheral circulation and the proliferation of those cells in the bone marrow. However, Emmel in his publication dealing with the aetiology of **Fowl Paralysis**, Leukaemia and Allied Conditions in Animals, Technical Bulletin 306, University of Florida (1936) states "In this study haemocytoblastosis is regarded as being designated by the presence of a variable number of immature blood cells of any type in the peripheral circulation." Emmel considers that this viewpoint does not differ materially from that of Johnson, but adds "In addition to immature forms of blood cells of any type, a variable number of degenerative forms of mature and immature blood cells are also found in the peripheral circulation"..... "Haemocytoblastosis is also characterised by a variable increase in the total number of white cells..... the erythrocyte count in active haemocytoblastosis is usually at least slightly under normal."

Emmel states that the condition in chickens may be caused by a number of agents, and that the stimulus necessary to arouse the haematopoietic system to action is not great. He considers it to be a variable process and has shown that it occurs after the intravenous injection of Salmonella bacteria, and following the oral exposure of birds to the same causal micro-organism during chronic intestinal parasitism. Dead bacteria (S. aertrycke) are also capable of inducing haemocytoblastosis when injected intravenously or intraperitoneally.

Perhaps the most important statement in the whole of Emmel's paper is that referable to the actual haemocytoblast cell itself in which he says, p.10 "The immature lymphocyte is in all probability an undifferentiated cell and one to which Johnson has referred to as the haemocytoblast." In an earlier Bulletin, No: 284, of the same University, August 1935, he describes the immature lymphocyte as a cell "closely resembling the small lymphocyte, sometimes slightly larger, but with cytoplasm not quite so basiphilic, and the nucleus is more vesicular, staining less intensively." Unfortunately, this is the only description of the cell given by Emmel and although he gives diagrams of most of the blood cells seen in haemocytoblastosis he fails to illustrate the particular cell itself.

As a fact, Emmel seems to have classified the blood cells to his own liking rather than conforming to recognised

methods, and indeed states, p.25, Bulletin 284 "Most of the numerous investigators who have made differential counts of blood smears from paralysed or leukaemic birds have used a different classification of blood cells. The classification used in this study is again different. While the author does not claim the classification used herein to be the best, it is simple and appears to give the best understanding of the blood picture in both natural and experimentally induced cases of fowl paralysis and leukaemia."

It would probably be more true to state that Emmel's method has caused the greatest confusion possible, because of its unnecessary divergence from accepted principles, and, that simple though it may be to the author himself, to others who have to rely on the description and diagrams given it is sufficiently confusing to cause a serious misunderstanding of the true blood picture.

In an endeavour to give a name to every cell appearing in the circulation, Emmel has failed to realise that the blood picture is only a mirror of the changes occurring elsewhere (notably in the marrow) and that the disease process is not really in the blood-stream but in the formative organs. This may appear harsh criticism, but the following notes will show clearly Emmel's confused ideas.

Bulletin 284, p.25, "Leukaemia - The author's conception of the blood picture in leukaemia is that the

fundamental change is one in which there is a degeneration of blood cells with a subsequent effort on the part of the bird to replace these degenerative cells with normal cells. The need for these normal cells is so urgent that immature erythrocytes, leucocytes or both are found in the circulating blood."

Emmel clearly considers the fundamental factor to operate on the cells in the blood rather than on the marrow.

p.26 - "Basophilic Polymorphonuclear Leucocyte (mast Cell) - This cell contains the characteristic polymorphonuclear nucleus, the cytoplasm containing closely packed basophilic granules."

Since the mast cell nucleus is never polymorphic but always monolobular and further that it is characterised by closely packed granules which overlie the nucleus it is improper to refer to the cell as having a typical polymorphonuclear nucleus.

p.27 - "Mature lymphocyte - No differentiation is made between the large and small lymphocyte. In some instances the large lymphocytes greatly outnumber the small lymphocytes. As yet no particular significance has been attached to any change in the numerical relation of these cells."

It can be seen that no appreciation is given to the genetic relationship between these two types of lymphocytes. Further, from a study of Emmel's drawings, although no measurements or scale are given, it is clear that what he describes as

the large lymphocyte is really only one of medium size. Then when actually describing a large lymphocyte he refers to this as the monocyte, and finally he introduces the "myelocyte" - "usually larger than the monocyte ..... the general structure of the cell closely resembles that of the monocyte."

Perhaps the greatest error in Emmel's description of the blood cells is that of the Vacuolar Lymphocyte - "The vacuolar lymphocyte is apparently a cell which McGowan (1926) has described as the fusiform cell. They often appear in groups."

From the complete histological description which follows, together with the diagram and the reference to McGowan's fusiform cell it is absolutely clear that Emmel is really describing the thrombocyte.

This has no relationship to the blood lymphocyte, and elsewhere in the paper Emmel writes "Thrombocytes have received no consideration in this paper."

From his description of the Immature Lymphocyte Emmel is describing an atypical, primary micro-erythroblast, which in the study in question is understood to represent the original stem cell or haemocytoblast.

The fact that Emmel has labelled the cells incorrectly and introduced terms unnecessarily does not mean that there is no such condition as Haemocytoblastosis. Indeed, it is only

too clear that the blood of chickens, under the stress of certain disease conditions, does show abnormalities apparently identical with those which Emmel has grouped collectively as haemocytoblastosis. However, his original description is not a happy one, for it is very indefinite, namely "a variable number of immature blood cells of any type." Since no indication is given as to the extent of the immaturity, nor yet how many types require to be involved before the picture can be termed haemocytoblastosis, the definition is clearly too vague.

ORIGINAL OBSERVATIONS ON HAEMOCYTOBLASTOSIS OF POULTRY

Haematology

The series of cases examined show in the blood three important features - (1) the presence of cells normally found in the bone marrow and never seen in the peripheral circulation of healthy birds, (2) degenerations involving all types of blood cells, and (3) a total white cell count, which, though frequently higher than normal, is not necessarily one of leucocytosis.

With the exception of this last statement, it will be seen that the general blood picture is similar to that described by Emmel. There is no suggestion, however, that the peripheral circulation contains numbers of "haemocytoblasts" or that there is proliferation of such cells in the marrow, as described by Johnson.

Although haemocytoblasts as such do not circulate, since the condition is one in which immature cells of all series appear peripherally, the term haemocytoblastosis does not appear to be wrong if used only in its descriptive sense.

Although the specific feature of haemocytoblastosis is the presence of immature cells circulating in the blood, the picture obtained is never that of true leukaemia, because neither myeloblasts nor lymphoblasts appear. The earliest granulocytes seen are premyelocytes - which in the fowl have a characteristic appearance - whilst on the lymphatic side of the picture there is nothing more immature than a large lymphocyte. However, as far as the red cells are concerned, erythroblasts commonly appear - often even the basic erythroblasts characterised by their deep blue cytoplasm. One may, therefore, consider the condition to be a mild form of erythroleucosis involving the whole reticulo-endothelial system, for on occasions pro-monocytes are also seen.

At this point it is advisable to consider the terminology used by Emmel in his various papers on the subject in order to show the corrections advocated -

Emmel's terms	Probably represents
Haemocytoblast	Atypical micro-erythroblast
Basophilic erythroblast	Primary erythroblast
Rod-bearing eosinophilic polymorphonuclear	Neutrophile ("polymorph") granulocyte

Emmel's terms	Probably represents
Granule-bearing eosinophilic polymorphonuclear	Eosinophile granulocyte
Polychrome erythrocyte	Immature (polychromasic) erythrocyte
Basophilic polymorphonuclear leucocyte	Basiphile granulocyte
Small lymphocyte	Small lymphocyte
Large lymphocyte	Medium sized lymphocyte
Monocyte	Large lymphocyte
Monocyte showing granules	Eosinophile metamyelocyte
Myelocyte	? Monocyte ?
Vacuolar lymphocyte (fusiform cell)	Thrombocyte
Immature lymphocyte	Atypical micro-erythroblast
Mature lymphocyte	Large or small lymphocyte
Premyelocyte	Premyelocyte
"Budding"	Cytoplasmic aggregations - notably in the lymphocytes.

The degenerations encountered involve all types of circulating cells and consist of:- vacuolation, lysis and clumping or budding of the cytoplasm; cell distortion generally - sometimes leading to rupture; nuclear pyknosis; basiphilia of the cytoplasm of the lymphocyte-monocyte series; atypical granules in the leucocytes; cell inclusions; formation of erythroplastids and distortions of the periphery of the erythrocyte nucleus leading to "notches."

An additional feature of the blood picture concerns the actual size of the various cells, for a marked feature of the present study has been their great variation and lack of uniformity. Anisocytosis, as is well-known, is a term usually restricted to excessive variations in the size of red cells, but its use in a wider sense might be advocated here, because there are just as great differences in size with nearly all the circulating cells - notably the granulocytes.

Poikilocytosis is not a common feature in diseases of avian blood, but erythrocytes more easily distorted than the majority and with pointed ends are to be seen in some cases of haemocytoblastosis.

The condition may, therefore, be visualised as one in which numbers of immature cells - normally retained in the marrow for maturation purposes - pass into the blood-stream hurriedly. Finding their new environment unsuitable for normal development purposes, they quickly show degenerative changes. It does not seem clear whether they are formed hurriedly and so, being of imperfect structure, they degenerate rapidly; or, whether their blood-stream environment - the plasma - should be considered unhealthy, resulting in the collapse of normal cells. The anisocytosis mentioned above is perhaps a point to be considered in favour of the theory that there is undue stimulation of the marrow. Such cells would almost certainly appear if there was

amitotic division of their immediate precursors - hence the finding of numbers of cells of all series of unequal size in the general circulation.

Perhaps the most difficult task is to decide when a given blood picture should be called one of haemocytoblastosis. How far must it differ from the normal? What of the finding of one or two premyelocytes, with perhaps a number of vacuolated lymphocytes? Fortunately, the answer is seldom difficult, for there is usually such a variety of well marked changes, few cases present themselves which leave any doubt as to the diagnosis of haemocytoblastosis.

There are over 20 features which may be noted in cases of haemocytoblastosis, therefore as a rough guide, if any ten or more of these are present, the diagnosis should be positive. There should be no doubt at all, of course, if both erythroblasts and premyelocytes are present in the same film.

An examination of large numbers of poultry bloods will show that certain of the features of the blood in haemocytoblastosi do occur normally - though to a very limited extent. Thus, erythrocyte degeneration, pyknosis, and some degrees of anisocytosis are apparently physiological, and although the stimulus for reticulosis (polychromasia) apparently need only be slight, the finding of an erythroblast of any type in the blood-stream is extremely rare. In making an estimate of the total leucocyte

count (by ascertaining the average number of white cells per field) if notes are also made of the number of degenerate cells (or other special features seen) then, after examining from 10-50 such microscopic fields - according to the case - a clear impression will be gained of the general blood picture.

The following are the chief points found in a haemocytoblastosis blood picture, contrasted with that from a healthy bird:-

Granulocytes	Normal	Haemocytoblastosis
Toxic granules	o	++
Ruptured cells	o - +	+++
Immature type granules	o - +	+++
Deficiency of granules	o - +	+++
"Anisocytosis"	o - +	+++
Immature types (pre-granular types; myelocytes; etc.)	o - +	++++

Lymphocytes	Normal	Haemocytoblastosis
Vacuoles in large variety	o	++
" " medium "	o - ++	+++
" " small "	o - +	+++
Very small varieties	o - +	+++
Azur granules or masses	o - +	++
"Turk cells	o - +	+++
"Budding" of cytoplasm	o - +	++++

Monocytes	Normal	Haemocytoblastosis
Vacuolated	o	o - +
"Turk-type	o	o - +
"Anisocytosis"	o	++

Erythrocytes	Normal	Haemocytoblastosis
Pyknosis	o - ++	++++
"Notches"	o - +	+++
"Anisocytosis"	o - ++	+++
Poikilocytosis	o - +	+++
"Plastid" formation	o	+++
Regeneration types	o - +	++++
Degeneration types	+	+++
Erythroblasts (polychromasic)	o - +	++++
Primary erythroblasts (basic)	o	++

Thrombocytes	Normal	Haemocytoblastosis
"Anisocytosis"	o - +	+++
"Poikilocytosis"	o - +	++
Condensation of the cytoplasm	o - +	+++
Pyknosis	o - ++	+++

HAEMOCYTOBLASTOSIS AND COCCIDIOSIS

A comparison of the haemocytoblastosis picture in ten chicks suffering from typical caecal coccidiosis contrasted with an equal number of cases involving the small intestines in pullets shows several interesting features.

In the duodenal coccidiosis cases there is a leucocytosis - averaging about 35,800 p.cm. - whereas in the caecal type there is a definite leucopenia with only about half this number present, i.e. 16,450 cells /c.mm. This is contrary to the experience of Emmel who found leucocytosis an invariable accompaniment of haemocytoblastosis. Since the number of leucocytes was often quite high, it must be assumed that there was operating in his cases a much more potent stimulus to the marrow. There is little doubt this would be due mainly to the nature of his experiments, i.e. that of inducing a Salmonella septicaemia; whereas contrasted with that the above cases were natural instances of early haemocytoblastosis.

In both types of coccidiosis, the myeloid response is best seen where the red cells are concerned for erythroblasts of all types occur together with other signs of active regeneration. At the same time, the destruction of mature erythrocytes in the blood-stream often appears increased, notably in caecal coccidiosis where there are often large numbers of nuclear remnants to be found.

Premyelocytes occur in more cases of caecal than duodenal coccidiosis, as do the pre-granular basiphile myelocytes, and there is also considerable variation in the size of the monocytes. In duodenal coccidiosis, many cases show a characteristic degeneration of the polymorphs, in which the rod-shaped granules appear as "clubs" - and, in some of the eosinophiles, the granules lose their natural round contour, to become semi-neutrophilic in type.

In this study, the lymphocytes show little of comparative value for Türk cells, vacuolations and "budding" are common to both types of coccidiosis. The cell described by Emmel as an immature lymphocyte - actually a small erythroblast with basic cytoplasm - occurs in about 45% of all cases.

No:	Degree of caecal coccidiosis	Age (weeks)	Notes	Degree of haemo-cytoblastosis
1	+++	7	pus +	+
2	+++	12	acute	+
3	+	6	post-cocc.	++
4	+++	4	acute	++
5	+++	6	chronic	+++
6	++	4	acute	+++
7	++	10	chronic	+++
8	++	4	acute	+++
9	+	12	post-cocc.	+++
10	None	6	-	++++

No:	Degree of caecal coccidiosis	Age (weeks)	Notes	Degree of haemo-cytoblastosis
11	+++	4	-	++++
12	++++	4½	-	++++
13	++++	4	-	++++
14	++	4	dying	++++
15	+	6	killed by cannibalism	++++

No:	Degree of duodenal coccidiosis	Age (weeks)	Notes	Degree of haemo-cytoblastosis
16	++	20	ascaridia ++	+
17	++	4½	caecal ++	++++
18	+	6	-	+++
19	+	12	davainea ++ capillaria + caecal +	+++
20	++	10	-	+++
21	+++	16	caecal +++	+++
22	++	18	advanced nephritis	+++
23	++++	12	davainea +	+++
24	++	11	heterakis + caecal ++++	++++
25	+++	10	caecal ++	++++
26	+++	10	-	++++

It will be seen from the accompanying Table that the degree of haemocytoblastosis does not appear to bear a direct relationship to the disease conditions, but this is somewhat to be expected because it is probable that haemocytoblastosis is an after effect of coccidiosis, rather than an accompaniment of the disease. The condition should, therefore, be visualised as a sequel to coccidiosis and not a direct symptom of the disease. The present study does not disclose whether uncomplicated coccidiosis alone can result in blood changes characteristic of haemocytoblastosis, or whether following an infestation by protozoa, bacteria require to gain access to the tissues - there to multiply, produce toxins and so stimulate the bone marrow. Emmel's theory is to the effect that the degree of haemocytoblastosis depends on the number of causal micro-organisms entering the blood-stream, their rate of entry, period over which they enter and potency. He considers that recovery from haemocytoblastosis is a slow process requiring as long as from 3-6 months, but he gives no figures for the "incubation period," although the growth curve of artificially induced cases in 3 weeks-old chicks shows the process to be well established after about 10-20 days.

Case 9 in the Table above is of special interest, because the post-mortem examination revealed no coccidiosis, although the blood picture was one of advanced haemocytoblastosis. This bird was taken from a flock where caecal coccidiosis had

been rife, and from the clinical standpoint it appeared quite healthy although perhaps slightly undersized. It is not improbable that the bird did suffer from a mild attack of coccidiosis, afterwards to recover. The development of haemocytoblastosis may have occurred as a direct result of the parasites, or only following the establishment of some bacterial focus. However, what is abundantly clear from the present study is that the effects of coccidiosis are not limited to the damage directly associated with the parasites as seen at post-mortem examination. Therefore, it is impossible to assess accurately the state of health of chicken following an outbreak of coccidiosis by noting their general appearance and weight without considering at the same time an examination of the blood cells.

HAEMOCYTOBLASTOSIS AND FOWL PARALYSIS

Emmel believes that haemocytoblastosis is an essential factor in the development of fowl paralysis although in itself he does not consider it will necessarily lead to paralysis - other factors require to be co-related with it.

Twenty-four cases of fowl paralysis have been studied and the degree of haemocytoblastosis noted. In fourteen instances, there were no blood changes considered sufficiently important to be classed as evidence of haemocytoblastosis, and in the remaining ten, only two showed advanced features, thus:-

Evidence as to haemocytoblastosis			24 fowl paralysis cases
Advanced	(+++)	=	2
Moderate	(++)	=	3
Slight	(+)	=	5
None	(-)	=	14

No relationship was noted between the degree of haemocytoblastosis and the extent or type of the fowl paralysis lesions, nor were the blood changes directly associated with the number of parasites present.

Contrasted with the previously mentioned cases of haemocytoblastosis (associated with coccidiosis) the stimulation of the marrow in fowl paralysis appears to be far less severe, for erythroblasts and premyelocytes were not numerous. Degenerative changes were marked, both in the erythrocytes and lymphocytes, particularly noteworthy being the large numbers of erythrocytes with indentations or pyknosis of the nucleus. A number of others were without nuclei - erythroplastids - and in every case, nuclear remnants were prominent. Lymphocytes with "budded" and vacuolated cytoplasm were common, as were Türk cells. The average white cell count was 37,500 cells/c.m. - a leucocytosis much the same as that noted in the cases of duodenal coccidiosis.

The main features are shown on the accompanying Tables.

Case Reference	314	225	333	280	128
Primary erythroblasts	-	-	-	+	+
Polychromasic erythroblasts	-	-	-	++	+
Regenerative R.b.cs.	++	-	-	+++	+++
Degenerative R.b.cs.	+	+	+	+	+
Nuclear Remnants	+	+	+	+	+
"Notches"	+++	-	+	+	-
"Plastids"	+	-	++	+	+
Pyknotic R.b.c.	++	-	++	+	-
Anisocytosis	++	++	-	++++	++
Thrombocytes	+++	-	-	-	++
"Budding"	+++	+	+++	+++	++
Vacuolation	++	-	++	++	-
"Turk" cells	-	-	+	-	+
Premyelocytes	++	-	+	-	-
Toxic granules	-	++	-	-	-
Basiphiles	-	-	-	+	-
Haemocytoblastosis	+++	+	++	+++	++
Leucocytes (000's)	37	68	34	57	10.5
Fowl paralysis	**Early** ++	Tumours ++++	F.P. ++	F.P. ++	Very early +
Parasites	++	-	++	-	++

Case Reference	201	298	294	219	299
Primary erythroblasts	-	-	-	-	-
Polychromasic erythroblasts	-	-	-	+	-
Regenerative R.b.cs.	+	-	-	++	+
Degenerative R.b.cs.	++	+	+	+++	+
Nuclear remnants	++	+	++	+++	+
"Notches"	+	-	++	-	-
"Plastids"	-	-	-	++	-
Pyknotic R.b.c.	-	+	-	+	-
Anisocytosis	-	+	-	-	-
Thrombocytes	-	++	-	-	-
"Budding"	+	+++	++	+	-
Vacuolation	+	+	+	+	+
"Turk cells	-	+	-	+	+
Premyelocytes	-	-	-	-	-
Toxic granules	-	-	-	-	-
Basiphiles	-	+	-	-	-
Haemocytoblastosis	+	+	+	++	+
Leucocytes (000's)	28	19.5	14	45	44
Fowl paralysis	F.P. +	advan-ced +++	advan-ced +++	eye +++	Eye +++ F.P. +
Parasites	-	+	-	++++	++++

HAEMOCYTOBLASTOSIS IN DAY OLD CHICKS

Hitherto, haemocytoblastosis has been regarded as a disease phenomenon, but examinations of day old chicks show that a similar process is operating at birth. The blood counts of 20 day-old chicks - some healthy and others too weak to break out of their shell - show changes identical with those recognised as haemocytoblastosis. As a fact, the response of the marrow is however greater where the red cells are concerned, for large numbers of primary erythroblasts are found in the circulation. There is great variation in the size of these, and in addition some of the lymphocytes show vacuoles or Türk-type cytoplasm and not a few of the basiphiles are of the early type. A few premyelocytes can also be found, so that a consideration of all these features of the blood shows clearly a picture very little removed from that of haemocytoblastosis. However, the total white cell count is greatly reduced so that a marked leucopenia exists.

The accompanying table shows the majority of the blood changes encountered in the physiological haemocytoblastosis of seven typical day-old chicks.

OTHER EXAMPLES OF HAEMOCYTOBLASTOSIS IN POULTRY

Following injections of a potent liver extract into pullets, there occurs a temporary stimulation of the bone marrow. This is reflected in the blood picture by the appearance of a

Haemocytoblastosis - Day old chicks

TABLE XXII

Reference	235	234	246	232	245	235	244
Primary erythroblasts	+	+	+	+++	+++	+	+
Polychromasic erythroblasts	++	++	+++	++++	+	-	++
Regenerative R.b.c.	+++	++++	++++	++++	++	+++	+++
Degenerative R.b.c.	++++	++	+	+++	++	+	+++
Nuclear remnants	++++	++	+	++	++	+	+
"Notches"	-	-	-	-	-	-	-
"Plastids"	+	+++	++++	+	+++	+++	+++++
Pyknotic R.b.c.	-	-	+	-	++	+	-
Anisocytosis	-	+	++	-	-	-	-
Platelets	-	-	-	-	-	-	-
Budding	-	-	-	-	-	-	-
Vacuolations	-	-	-	-	-	-	-
"Turk cells	++	+	+++	+	-	-	++
Premyelocytes	-	+	-	-	-	-	-
Toxic granules	++	-	-	++	-	-	++
Basiphiles	-	-	-	-	+	-	-
W.b.cs.	9.2	9.0	9.5	8.5	55.00	99.55	66.88

small number of erythroblasts - both primary and polychromasic- and there are also other regenerative signs. Immature leucocytes or degenerative forms may also be found, therefore this response may be considered to represent an early type of haemocytoblastosis. Similarly, young chicks aged 2-4 days suffering from the effects of severe chilling show blood changes typical of haemocytoblastosis.

An 18-day old chick, a survivor from an outbreak of Bacillary White Diarrhoea, has also been found to show in the blood typical signs of haemocytoblastosis. Erythroblasts and erythroplastids were present, whilst some other red cells were immature or with pyknotic nuclei. Monocytes, lymphocytes and a few erythrocytes were vacuolated, as was an occasional neutrophile.

Here then was an example of haemocytoblastosis showing early red cells and degenerate red and white cells, but no early leucocytes.

As a contrast, a bird suffering from Fowl Pox showed numerous premyelocytes and degenerative leucocytes, but no anaemic features.

Finally, typical changes representative of advanced haemocytoblastosis have been noted in a fowl suffering from Leukaemia (myeloid) with dropsy, and also in Blackhead in turkeys.

SUMMARY OF OBSERVATIONS ON HAEMOCYTOBLASTOSIS IN POULTRY

From a full consideration of the foregoing statements, it is possible to summarise the available knowledge as follows:-

So-called "haemocytoblastosis" is an example of myeloid stimulation in poultry. It is characterised (in its advanced typical form) by the finding of immature or degenerate red and white cells in the peripheral blood circulation. It is non-specific, occurring in chicken of all ages, breeds and in both sexes; and, although commonly encountered in the domestic hen, it has also been observed in the turkey.

Contrary to the experience of Emmel, haemocytoblastosis is not always associated with an increase in the total number of white cells, nor is it necessarily of pathogenic origin, for identical changes are seen in the blood of healthy day old chicks. Apart from this physiological occurrence of haemocytoblastosis it has been observed in chilled chicks, following injections of liver extract, and in diseases of virus, bacterial and parasitic origins, and although erythroblasts are nearly always seen in typical cases of haemocytoblastosis, the leucocyte response is far less intense, for neither myeloblasts nor lymphoblasts appear in the circulation but premyelocytes are always found when the process is advanced.

All stages between the typical haemocytoblastosis picture of Emmel and normal blood counts have been recorded, and therefore haemocytoblastosis is considered to represent a type of myeloid response in poultry, and not a disease *per se*.

It has not been proved in the present series of cases that haemocytoblastosis is an essential factor in the development of fowl paralysis, for it does not appear as a constant finding in blood examinations of birds suffering from this disease, but it would rather appear to represent a post-coccidial manifestation. Since coccidiosis, in some form or degree, precedes fowl paralysis in large numbers of cases, the association between haemocytoblastosis and fowl paralysis is probably more directly connected with the damage of the coccidial parasites than with any specific neurotropic virus sometimes postulated as the specific cause of *neuro-lymphomatosis-gallinarum*.

In view of the greater response of that portion of the bone marrow associated with erythrocytopoiesis it is probably more correct to refer to the condition, in its typical form, as *erythroblastosis*.

The percentage distribution of the main features characteristic of haemocytoblastosis in new-born chicks and in cases of coccidiosis and fowl paralysis is shown on the accompanying Table.

HAEMOCYTOBLASTOSIS IN THE DOMESTIC HEN

	Normal day old chicks %	Caecal coccidiosis %	Duodenal coccidiosis %	Fowl paralysis %
Primary erythroblasts	100	60	70	20
Polychromasic erythroblasts	100	70	80	30
Polychromasic erythrocytes	100	70	80	60
Nuclear remnants	100	90	100	100
Pyknosis	40	60	70	50
Anisocytosis	40	40	50	50
Erythroplastids	100	80	60	50
"Notches"	0	50	40	50
Türk cells	70	70	60	50
Vacuolated lymphocytes	0	40	50	80
Haemocytoblasts	40	30	60	20
Leucocytosis	0	70	20	60

GENERAL SUMMARY

Part I

A technique for the examination of the blood cells of animals for use on the farm or in the laboratory has been established.

Hitherto, haematology has not played a great part in veterinary practice, but a study of the blood cells in animal diseases is a practical procedure and one which can prove useful as an aid both to diagnosis and prognosis.

There are few occasions in which the blood picture is specific for any one disease, but a careful examination of the differential count combined with haemoglobin and total cell counts may be of value to veterinary practitioners. A method has been suggested for the estimation of the leucocytes and except where fine degrees of anaemia are of importance - such as in tuberculosis - total red cell counts are not usually a necessity.

There are no primary anaemias in the animals examined and leukaemias - comparable with those of man in which the earliest forms of blood cells are found in the general circulation - are uncommon.

The staining method employed has been that of Pappenheim, modified by Piney, using May-grunvald, Pancrom and methyl-green-orange-G compounds. The cells all stain clearly and in the fowl especially the necessity for supra-vital

staining is in many instances eliminated. Automatic diluting pipettes are an improvement over the usual Thoma type and for leucocyte counts in the domestic hen, distilled water causes lysis of the erythrocytes, leaving the white cells available for counting purposes.

The investigation has necessitated the examination of some 90,000 - 100,000 leucocytes, for a differential count was made on over 300 animals bloods - 90 of which were from cattle.

Part II

The blood cells of cattle have been studied at all ages and their histological appearances noted both in supra-vital and panoptically stained films.

Attention has been paid to the blood changes following birth and of the alterations to the blood picture as maturity is reached.

A special study has been made of the differential count in health and disease and also of the Arneth, Polynuclear and Schilling methods for the computation of the nucleus of the granulocytes. The granules of the polymorphs are difficult to stain and the distribution of the oxychromatin of the nucleus is such that false segmentation appears common and therefore the rendering of a polynuclear count may be difficult.

A study of the lymphocytes has shown that they too should be classified and the following formula is useful for clinical purposes:-

$$LL + \frac{ML}{2} : \frac{ML}{2} + SL$$

The blood changes occurring in the following diseases have been noted and discussed:- tuberculosis, Johne's disease, certain pyogenic infections, Bovine pasteurellosis, actinomycosis (actinobacillosis) and contagious abortion.

The blood picture in this latter disease presents an interesting comparison with that of Undulant Fever, and in connection with tuberculosis there is some evidence to show that the differential count may be valuable in deciding whether an indefinite reactor to the tuberculin test is really infected or otherwise.

The effect of Prontosil rubrum and sulphanilamide on the blood picture of cattle has been recorded. A safe dose of either preparation for the dairy cow is 50 grammes twice daily - the limit of tolerance having been reached after the giving of about 1,250 x 0.5 gramme tablets.

Part III

The blood cells of the domestic hen have been studied and their histological characters noted and discussed.

A study has been made of erythroplastid formation in the hen and of its relationship to erythropoiesis - this is of particular interest in relation to the question of the extrusion of the nucleus of the mammalian normoblast.

The suggestion of McGowan that the avian thrombocyte is an involuted erythrocyte receives support from observations recorded in this thesis.

The Guttadiaphot (Schilling) test cannot be applied to poultry, for in health their blood gives a marked positive reaction.

The blood picture at hatching time is characteristic in birds (erythroblastosis foetalis) and a study has been made of the changes which occur during the first few days of life in the chick.

Alterations in the erythrocytes show that intra-vascular haemolysis is a normal method for the destruction of red cells in poultry.

An attempt to use the differential count as an aid to the standardisation of liver extracts was not successful, but as the subject is of importance further studies on the lines indicated may not be wasted.

The blood picture in the following diseases has been studied:- tuberculosis, fowl pox, fowl paralysis and coccidiosis. Special attention has been paid to the leucocyte formula in fowl paralysis and coccidiosis and also to Emmel's suggestion that haemocytoblastosis is a normal accompaniment of the former disease. The present investigation does not confirm this belief, but shows that haemocytoblastosis is frequently a post-coccidiosis phenomenon, thus showing an even greater connecting link between the two diseases than usually accepted.

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TABLE I

NORMAL CALVES

Ref.	Total WBC's	Differential Count								% HB	AGE
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
253	9,500	83.6	0.3	0.4	12.7	2.7	0.0	15.4	0.3	84	4 hrs.
154	-	77.0	0.0	0.0	6.0	11.5	3.5	21.0	0.2	72	8 hrs.
144	-	67.6	0.0	0.4	12.3	8.3	3.7	24.3	7.7	-	8 hrs.
230	7,200	71.7	0.0	0.3	14.3	8.7	3.0	26.0	2.0	88	16 hrs.
308	14,200	66.0	0.4	2.7	20.0	6.3	2.3	28.6	2.3	89	36 hrs.
166	9,350	59.3	0.3	0.0	9.4	13.7	11.7	34.8	5.6	90	41 hrs.
254	13,500	53.0	0.0	1.0	15.0	17.0	11.0	43.0	3.0	81	5½ days
219	-	48.0	0.0	1.3	6.3	29.3	14.7	50.3	0.4	-	5 days
175	-	42.6	0.7	0.7	3.7	42.0	8.3	54.0	2.0	-	7 days
148	9,000	41.7	0.0	1.0	22.0	26.0	4.7	52.7	4.6	72	10 days
229	13,500	46.3	1.0	0.7	19.3	17.3	11.0	47.6	4.4	81	14 days
401	-	10.0	0.0	0.0	7.5	22.5	57.5	87.5	2.5	-	9 weeks
399	-	10.4	0.8	0.8	8.0	37.0	35.0	80.0	8.0	-	3 mths.
398	4,800	30.0	2.0	0.0	10.0	31.0	30.0	71.0	1.0	-	3 mths.

NORMAL BOVINES **TABLE** YEARLINGS, HEIFERS AND COWS.

II

Ref.	Total WBC's	Differential Count								RBC'S	
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
Yearlings											
46	16,100	25.4	3.3	0.3	14.7	30.3	21.0	66.0	5.0	16,600,000	
47	16,900	12.3	0.4	0.0	14.0	40.0	30.0	84.0	3.3	8,576,000	
48	12,800	21.6	1.0	1.0	8.7	23.7	38.0	70.4	6.0	11,008,000	
49	17,400	26.3	4.3	0.4	18.3	24.3	20.0	62.7	6.3	10,000,000	
50	-	30.0	0.7	1.3	7.0	24.0	31.0	62.0	6.0	5,240,000	
Heifers										% HB	W.M.I.
183	9,000	42.0	6.5	0.0	7.5	20.0	22.0	49.5	2.0	82	1.70
184	9,000	21.3	18.7	2.0	6.3	28.3	21.7	56.3	1.7	74	1.32
185	9,000	25.3	13.3	2.0	5.7	28.7	24.0	58.4	1.0	70	1.32
186	12,500	31.3	23.0	1.0	14.7	16.0	11.3	42.0	2.7	70	1.42
Dairy cows										W.M.Index	
210	-	22.7	9.0	1.3	13.0	39.0	13.0	65.0	2.0	1.46	
212	-	37.0	16.0	0.0	21.0	14.0	10.0	45.0	2.0	1.32	
213	-	33.3	30.0	0.4	5.3	22.3	7.7	35.3	0.7	1.47	
214	-	33.7	9.7	1.0	17.3	29.0	9.3	55.6	0.0	1.33	
215	-	17.0	24.6	0.7	13.7	36.7	7.0	57.4	0.3	1.40	

NORMAL BOVINES

TABLE III

NEWLY CALVED COWS, BULLOCKS AND BULL

Ref.	Total WBC's	Differential Count									
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
Newly calved cows										HB%	W.M.I.
231	6,500	38.0	7.3	1.0	23.7	16.3	5.3	45.3	8.4	85	1.41
251	8,500	48.0	7.0	0.0	25.0	15.3	2.0	42.3	2.7	75	1.44
307	7,400	33.0	11.0	2.3	18.7	14.7	19.0	52.4	1.3	102	1.37
Bull-ocks										RBC'S (0000)	HB%
165	16,800	18.3	10.3	0.3	6.0	33.0	31.0	70.0	1.0	618	108
196	6,600	23.0	6.5	1.5	12.0	27.5	25.5	64.5	4.5	754	120
197	9,900	40.3	1.7	0.0	6.0	15.0	35.0	56.0	2.0	752	110
198	-	40.7	2.0	0.0	9.0	22.3	24.3	55.6	1.7	-	-
200	18,300	25.0	3.7	0.0	5.7	29.3	34.0	69.0	2.3	552	90
201	9,400	32.7	5.3	0.3	3.8	21.8	34.1	59.7	2.0	548	110
203	15,333	32.3	2.3	0.0	7.7	27.7	28.7	64.1	1.3	651	115
204	11,970	39.7	6.7	0.3	9.3	25.3	16.7	51.3	2.0	768	90
205	12,600	26.7	4.0	0.3	7.0	23.7	37.0	67.7	1.3	860	105
207	24,333	36.7	10.7	0.6	2.3	24.7	22.0	49.0	3.0	705	120
208	11,933	25.7	9.7	0.3	2.7	36.3	22.3	61.3	3.0	798	100
Bull 2 yrs.										W.M.I.	HB%
	5,600	22.0	6.5	0.0	53.5	10.5	3.0	67.0	4.5	1.36	82

DISEASED CATTLE **TABLE IV** TUBERCULOSIS AND JOHNE'S DISEASE

Ref.	Total WBC's	Differential Count								Disease
		Ne	Eo	Ba	Lymphocytes				Mono	
					Large	Medium	Small	Total		
360A	-	4.0	0.0	0.2	9.4	27.2	58.2	94.8	1.0	Tubercu-losis
360B	-	6.0	0.0	0.3	14.7	27.7	50.7	93.0	0.6	"
209	5,000	26.0	6.0	4.0	-	-	-	42.0	22.0	"
211	2,500	47.0	6.0	5.0	15.0	22.0	2.0	39.0	3.0	"
202	9,533	40.7	11.3	0.3	3.3	19.0	22.3	44.7	3.0	"
1814	-	22.0	3.7	0.3	6.3	37.7	18.0	62.0	10.0	Johne's disease
1813	8,000	25.0	7.25	0.25	2.5	28.5	36.5	67.5	0.0	"
1812	-	20.6	8.0	0.0	7.3	19.3	42.4	69.0	2.4	"
1811	25,000	63.5	1.0	1.0	8.0	4.0	3.5	15.5	19.0	"
179A	6,500	27.5	22.0	1.5	14.0	19.5	14.5	48.0	1.0	Normal
179B	8,500	37.3	8.0	1.0	10.0	18.0	24.0	52.0	1.7	T.T.tested
292A	8,500	29.0	9.0	1.3	23.0	18.7	17.0	58.7	2.0	Normal
292B	9,000	32.0	4.0	0.7	24.0	19.7	13.6	57.3	6.0	T.T.tested 48th.hour
292C	9,000	21.3	6.0	1.0	27.7	37.3	3.7	68.7	3.0	72nd.hour
248	9,000	14.3	6.7	1.0	26.3	37.7	10.7	74.7	3.3	Failed T.T.test
255	13,500	32.0	6.0	0.7	39.3	16.7	4.3	60.3	1.0	Failed T.T.test
287	9,000	23.0	11.7	0.3	17.0	21.7	24.0	62.7	2.3	Failed T.T.test

DISEASED CATTLE **TABLE V** BACTERIAL INFECTIONS

Ref.	Total WBC's	Differential Count								Condition
		Ne	Eo	Ba	Lymphocytes				Mono	
					Large	Medium	Small	Total		
203	12,000	66.7	0.3	0.3	5.0	16.7	8.0	29.7	3.0	Bovine pasteur-ellosis
204	5,000	16.5	7.0	1.0	7.5	28.0	40.0	75.5	0.0	"
205	7,500	20.0	15.0	0.0	10.0	32.0	20.0	62.0	3.0	"
270	5,000	26.0	13.5	3.5	14.5	16.5	25.0	56.0	1.0	"
271	15,800	40.3	11.0	0.7	19.0	9.3	17.7	46.0	2.0	"
272	8,200	40.0	11.0	1.0	10.0	16.0	20.0	46.0	2.0	"
123	8,000	40.3	6.0	0.7	9.6	14.3	28.7	52.6	0.4	Leucorr-hoea
122	7,000	20.0	12.0	0.7	10.0	18.0	38.0	66.0	0.3	"
309	7,200	6.7	5.3	0.4	18.3	37.3	32.0	87.6	0.0	"
306	8,500	35.0	22.0	1.0	9.0	23.0	10.0	42.0	0.0	Metritis
217	12,000	65.7	6.3	0.7	16.7	9.3	1.3	27.3	0.0	Mastitis
153	6,800	64.0	1.0	0.0	9.0	14.0	4.0	27.0	8.0	Mammitis
305	9,000	47.0	1.0	0.0	19.0	17.0	15.0	51.0	1.0	Toxaemia
340	13,800	28.3	8.0	2.0	19.7	28.0	7.7	55.4	6.3	Actino-bacillosis

DISEASED CATTLE **TABLE** VI CONTAGIOUS ABORTION

Ref.	Total WBC's	Differential Count								% HB	Age
		Ne	Eo	Ba	Lymphocytes: Large	Lymphocytes: Medium	Lymphocytes: Small	Lymphocytes: Total	Mono		
174	9,000	26.0	25.0	0.0	7.0	22.0	17.0	46.0	3.0	70	3½
175	9,200	25.3	19.0	1.7	10.0	28.0	11.3	49.3	4.7	73	4½
176	6,800	12.0	18.0	0.5	7.5	42.5	17.0	67.0	2.5	70	3½
177	4,500	38.0	12.0	0.0	14.0	31.6	2.7	48.3	1.7	72	5
178	-	17.5	20.0	1.5	7.5	33.5	19.0	60.0	1.0	78	4½
180	6,000	21.0	31.5	2.5	16.0	20.5	6.5	43.0	2.0	70	7½
182	12,800	14.3	25.3	1.0	15.0	29.3	9.7	54.0	5.4	78	3½
187	7,800	21.3	23.0	1.7	13.0	26.0	14.3	53.3	0.7	80	4½
188	4,800	26.5	8.5	2.5	10.0	41.0	10.5	61.5	1.0	78	4
227	13,500	56.0	9.3	1.0	9.3	10.0	9.7	29.0	4.7	78	5
175	9,200	25.3	19.0	1.7	10.0	28.0	11.3	49.3	4.7	Before treatment	
189	6,800	26.0	24.0	1.0	4.0	24.0	7.0	45.0	4.0	After one dose.	
191	9,000	50.0	16.7	1.7	3.0	16.7	9.3	29.0	2.6	After five doses.	
215	8,200	50.7	18.0	1.0	8.3	13.0	7.0	28.3	2.0	Five days later.	
222	9,000	54.3	6.3	0.0	15.7	10.7	5.0	31.4	8.0	After 150 grammes.	
250	9,000	22.3	7.0	4.7	34.0	19.3	8.7	62.0	4.0	One week later.	

POULTRY **TABLE** VII NORMAL CHICKS

Ref.	Total WBC's	Differential Count								% HB.	Age.
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
141	9,000	82.0	0.0	0.0	2.0	2.0	10.0	14.0	4.0	-	1 day
244	6,800	74.0	1.0	1.0	4.0	10.0	9.0	23.0	1.0	72	"
234	9,000	65.0	1.5	5.5	7.0	14.0	6.0	27.0	1.0	85	"
233	9,500	68.0	0.0	3.0	3.0	16.0	8.0	27.0	2.0	68	"
245	5,000	66.0	2.0	3.0	3.0	12.0	14.0	29.0	0.0	72	"
232	8,500	60.0	3.0	4.0	2.0	16.0	13.0	31.0	1.0	76	"
246	9,500	57.0	0.5	1.0	9.0	16.5	15.0	40.5	1.0	76	"
236	9,000	54.0	1.5	5.5	5.5	20.5	13.0	39.0	0.0	80	"
235	9,200	53.0	3.0	4.0	4.0	17.5	16.5	38.0	2.0	60	"
143	6,500	52.0	0.0	8.0	8.0	8.0	24.0	40.0	0.0	-	2 days
147	8,000	50.0	6.0	9.0	3.0	30.0	2.0	35.0	0.0	70	2½ "
223	17,800	54.7	1.0	2.3	6.3	8.0	27.0	41.3	0.7	62	10 "
164	2,800	86.0	0.0	0.0	2.0	4.0	4.0	10.0	4.0	60	In-shell
160	2,000	64.0	6.0	9.0	9.0	6.0	6.0	21.0	0.0	60	In-shell
163	9,000	49.0	4.0	5.0	7.0	17.0	16.0	40.0	2.0	54	In-shell
162	-	44.0	6.0	4.0	12.0	22.0	10.0	44.0	2.0	60	In-shell
239	3,500	15.0	0.0	15.0	12.0	36.0	18.0	66.0	4.0	72	In-shell

NORMAL POULTRY **TABLE VIII** PULLETS AND HENS IN HEALTH

Ref.	RBC's (000) Total WBC's	Differential Count								
		Ne	Eo	Ba	Lymphocytes				Mono	
					Large	Medium	Small	Total		
1168	3,230	17.7	3.0	2.7	8.3		68.0	76.3	0.0	
1170	3,715	30.5	0.75	2.25	23.5	-	43.0	66.5	0.0	
1171	3,220	22.7	1.3	0.9	13.3	-	61.8	75.1	0.0	
1173	3,500	16.0	3.7	3.0	10.0	-	67.3	77.3	0.0	
1203	3,520	19.3	7.3	5.3	9.3	-	58.4	67.7	0.4	
1204	-	30.0	6.7	1.7	15.0	-	46.3	61.3	0.3	
1205	3,512	16.3	1.7	3.7	9.3		69.0	78.3	0.0	
1206	3,160	22.3	2.0	2.7	17.3		55.3	72.7	0.3	
1207	-	32.0	0.5	1.5	7.5		58.5	66.0	0.0	
1208	-	16.0	1.0	3.7	10.6		67.0	77.6	1.7	
1209	-	36.3	0.3	2.7	10.3		50.3	60.7	0.0	
										HB %
114	9,000	27.0	1.0	1.0	4.3	5.3	60.4	70.0	1.0	78 Cock

NORMAL POULTRY **TABLE IX** PULLETS AND HENS IN HEALTH

Ref.	Total WBC's	Differential Count								% HB	
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
220	27,000	31.0	2.0	4.0	1.3	7.7	54.0	63.0	0.0	74	Broody
261	18,200	25.0	4.0	2.7	13.3	8.3	46.3	67.9	0.4	87	"
278	27,000	27.0	1.7	3.3	7.3	17.0	43.7	68.0	0.0	79	"
	RBC's (000)										
685	2,800	14.0	20.3	1.0	4.7	-	60.0	64.7	0.0		
688	3,136	16.0	3.0	1.0	17.6	-	61.7	79.3	0.7		
1049	3,312	11.0	5.3	1.7	8.0	-	74.0	82.0	0.0		
1098	2,888	15.0	1.0	1.7	7.7	-	74.7	82.3	0.0		
1119	2,392	17.0	5.3	3.3	11.3	-	62.7	74.0	0.4		
1129	2,500	11.0	4.7	1.3	8.3	-	74.7	83.0	0.0		
1132	3,256	18.6	2.7	4.7	11.7	-	62.3	74.0	0.0		
1164	2,750	17.7	3.0	0.7	8.7	-	69.3	78.0	0.6		
1165	3,300	15.7	3.3	2.0	9.0	-	68.0	77.0	2.0		
1166	2,770	25.3	1.7	3.0	16.0	-	54.0	70.0	0.0		
1167	3,180	19.0	4.3	2.3	7.7	-	66.7	74.4	0.0		

DISEASED POULTRY **TABLE X** TUBERCULOSIS

Ref.	Total WBC's	Differential Count								% HB	Clinical Notes
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
114	9,000	27.0	1.0	1.0	4.3	5.3	60.4	70.0	1.0	78	Normal
226	24,500	21.0	0.3	4.7	10.0	14.3	49.0	73.3	0.7	80	Pre-injection
252	25,000	20.6	1.7	1.7	10.0	22.3	43.0	75.3	0.7	80	6 days later
273	21,000	24.7	2.0	5.7	10.0	22.0	34.6	66.6	1.0	80	T.test negative
289	27,000	23.0	0.3	2.3	15.0	33.7	24.7	73.4	1.0	73	3 days later
297	17,800	20.7	1.6	3.3	13.7	11.7	48.3	73.7	0.7	87	18 days after injection
302	28,000	50.7	1.7	1.7	11.7	15.3	18.6	45.6	0.3	84	T.test positive.
341	32,000	20.6	1.0	2.3	11.0	20.7	43.7	75.4	0.7	77	6 weeks later
346	15,000	38.0	2.0	1.0	16.0	22.0	21.0	59.0	0.0	74	1 week later
173	38,500	40.3	3.2	5.0	27.7	10.0	13.8	51.5	0.0	60	Typical case
221	30,000	54.0	2.3	6.0	19.7	2.7	15.3	37.7	0.0	60	Advanced lesions
126	45,000	62.5	1.5	1.0	15.2	1.3	18.5	35.0	0.0	46	Dying

DISEASED POULTRY **TABLE** XI B.W.D. FOWL POX.

Ref.	Total WBC's	Differential Count								% HB	
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
124	95,000	71.3	0.7	0.3	15.3	3.7	7.7	26.7	1.0	50	Chick
125	7,000	71.0	0.0	2.0	6.0	6.0	14.0	26.0	1.0	45	Chick
21	-	13.3	0.7	0.3	14.7	-	71.0	85.7	0.0	Carrier hen	
22	-	44.3	0.7	2.0	15.7	-	37.3	53.0	0.0	"	
23	-	34.3	0.8	1.3	19.3	-	42.2	61.5	2.0	"	
24	-	42.3	1.0	4.7	15.3	-	36.3	51.6	0.4	"	
25	-	41.3	1.0	1.7	10.7	-	45.0	55.7	0.3	"	
1088	-	50.0	1.0	0.5	20.0	-	27.5	47.5	1.0	Fowl Pox	
2511	-	59.7	1.3	1.5	5.2	-	30.0	35.2	2.3	"	"
4231	-	12.3	2.7	1.3	23.0	-	59.0	82.0	1.7	"	"
26	-	30.3	1.0	0.3	18.7	-	49.7	68.4	0.0	Pigeon Pox	
27	-	62.0	0.3	2.7	-	-	-	34.0	0.0	"	
28	-	80.2	0.7	0.0	15.0	-	4.2	19.1	0.0	"	
29	-	84.0	0.3	0.7	6.0	-	8.3	14.3	0.7	"	
30	-	39.7	1.3	4.7	11.7	-	41.7	53.3	1.0	"	

DOMESTIC HEN **TABLE XII** LIVER EXTRACT INJECTIONS

Ref.	Total WBC's	Differential Count								% HB	Liver injections.
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
Pullet											
323	23,000	23.75	2.0	4.25	4.25	19.0	46.75	70.0	0.0	62	Before
324	39,000	27.0	2.7	3.3	3.0	8.7	55.3	67.0	0.0	70	After 26 hrs.
Hen											
260	27,200	24.7	5.3	3.3	13.3	7.7	45.3	66.3	0.4	87	Before
262	23,000	19.3	5.0	5.0	6.3	21.7	42.0	70.0	0.7	85	After 22 hrs.
274	27,800	32.0	8.3	4.0	4.3	6.0	45.4	55.7	0.0	88	After 48 hrs.
291	26,800	20.7	9.3	3.7	9.0	7.3	49.7	66.0	0.3	87	After 72 hrs.
Pullet in lay											
300	21,000	44.3	1.0	1.0	2.7	5.0	46.0	53.7	0.0	70	Before
304	18,000	29.0	0.3	4.3	5.7	12.3	48.0	66.0	0.4	65	After 20 hrs.
316	-	47.0	0.5	1.5	3.5	11.5	36.0	51.0	0.0	75	After 96 hrs.
Young pigeon											
320	28,800	48.0	1.0	0.3	7.3	25.7	17.7	50.7	0.0	59	Before
325	38,000	46.0	0.0	1.0	3.3	27.7	21.7	52.7	0.3	64	After 24 hrs.

DISEASED POULTRY **TABLE XIII** FOWL PARALYSIS

Ref.	Total WBC's	Differential Count								Type of Lesion	% HB
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
116	36,000	20.0	8.0	6.7	7.7	7.0	50.3	65.0	0.3	Iritis	50
118	27,000	13.0	5.3	0.4	7.3	14.7	59.3	81.3	1.0	Leg	60
127	48,000	32.5	3.3	4.75	13.7	4.5	41.3	59.5	0.25	Legs	88
128	10,500	14.0	3.3	3.7	19.3	19.7	39.0	78.0	1.0	Early	86
129	25,000	43.0	2.0	2.2	14.0	4.8	34.0	52.8	0.0	Legs	75
146	28,000	50.7	6.3	3.7	9.7	4.6	24.0	38.3	1.0	Advan-ced	62
168	21,000	44.8	2.5	4.7	16.3	10.0	21.2	47.5	0.5	Legs	50
217	27,800	48.7	0.3	0.3	20.7	6.7	21.3	48.7	2.0	Legs	86
219	45,000	9.0	0.3	0.7	3.7	8.0	78.3	90.0	0.0	Iritis	70
225	68,000	85.3	0.3	0.4	5.0	0.3	8.3	13.6	0.4	Tumours	50
262A	-	24.0	0.5	0.0	1.0	8.0	62.0	71.0	0.0	Legs	-
262B	-	14.7	2.7	1.6	4.0	3.3	73.7	81.0	0.0	Legs	-
262C	-	22.0	3.3	5.0	5.3	-	63.0	68.3	1.3		-
280	57,000	41.3	2.7	2.3	12.7	12.7	27.0	52.4	1.3	Legs	65
314	37,000	16.0	2.0	3.0	5.0	15.0	59.0	79.0	0.0	Early	82
333	34,000	22.0	2.3	3.0	8.0	20.0	44.3	72.3	0.3	Legs	70
121	85,000	24.7	1.7	1.3	6.7	19.6	45.7	72.0	0.3	Iritis	65
201	28,000	24.7	2.7	4.3	7.3	12.7	48.3	68.3	0.0	Early	-
218	27,200	26.3	17.7	2.4	12.0	15.3	25.3	52.6	1.0	Iritis	59
276	18,000	17.0	5.0	3.0	17.0	52.0	6.0	75.0	0.0	Iritis	65

DISEASED POULTRY **TABLE** XIV FOWL PARALYSIS (CONT.)

Ref.	Total WBC's	Differential Count								Type of Lesion	% HB
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
277	18,500	40.3	26.0	4.7	7.7	9.7	11.6	29.0	0.0	Iritis	49
294	14,000	39.7	3.3	2.3	11.7	21.3	20.7	53.7	1.0	Nerve	90
298	19,500	31.7	3.0	4.3	12.0	13.7	35.3	61.0	0.0	Leg	91
299	44,000	15.0	3.0	2.3	8.7	8.7	62.0	79.4	0.3	Iritis	62
310	42,000	24.7	1.7	4.0	8.3	11.0	49.7	69.0	0.6	Leg	68
319	40,000	24.0	4.3	6.7	9.0	9.7	46.3	65.0	0.0	Leg	78
2311		35.0	2.5	4.0	3.0	15.0	40.0	58.0	0.5	Leg	
2312		36.0	3.7	2.0	5.3	12.0	41.0	58.3	0.0		
2313		40.0	2.7	2.7	6.0	10.6	38.0	54.6	0.0		
2314		33.7	4.0	1.7	8.0	11.0	41.6	60.6	0.0		
2315		40.3	1.3	3.7	10.0	9.7	35.0	54.7	0.0		
2316		36.3	1.7	4.7	8.3	12.7	36.3	57.3	0.0		
106	50,000	30.5	2.0	6.0	7.5	4.5	47.0	59.0	2.5	Early	-
107	40,000	28.3	3.3	4.5	15.7	5.0	42.2	62.9	1.0	Early	-

DISEASED POULTRY **TABLE** WORM INFESTATIONS

XV

Ref.	Total WBC's	Differential Count								% HB	
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
286	40,000	9.7	5.0	2.0	21.7	14.0	46.6	82.3	1.0	86	DCCO
200	35,800	67.0	0.7	0.0	14.0	7.0	11.3	32.3	0.0	84	DAH
198	44,200	9.7	31.7	1.6	10.3	4.3	42.4	57.0	0.0	75	DAVAS
197	31,500	17.7	15.7	5.0	8.7	8.3	44.6	61.6	0.0	80	MCI
152	27,000	82.0	0.0	2.0	4.0	4.0	8.0	16.0	0.0	74	DAVCO
133	26,500	8.3	0.7	3.7	6.0	10.7	69.6	86.3	1.0	78	HET
132	20,000	11.0	0.0	1.4	9.0	22.7	54.3	86.0	1.6	78	ASHT
131	-	50.3	2.0	2.0	3.7	4.0	38.0	45.7	0.0	-	AMO
105	35,000	44.3	5.7	8.3	4.7	2.0	35.0	41.7	0.0	-	DAV
105D	-	48.0	4.3	9.7	25.3	4.3	8.0	37.6	3.4	55	DAV

INFESTATIONS

DAV - Davainea.

DAH - Davainea, Ascaridia and Heterakis.

DAVAS - Davainea and Ascaridia.

MCI - Mixed cestode infestation.

DAVCO - Davainea and coccidia.

AMO - Amoebotaenia.

HET - Heterakis.

ASHT - Ascaridia and Heterakis.

DCCO - Davainea, Capillaria and coccidia.

DISEASED POULTRY

TABLE XVI

ANAEMIA, LEUKAEMIA AND CHILL

Ref.	Total WBC's	Differential Count								
		Ne	Eo	Ba	Lymphocytes				Mono	
					Large	Medium	Small	Total		
FA1	-	54.5	0.0	0.5	9.5	-	35.0	44.5	0.5	Anaemia
FA2	-	73.0	0.7	1.3	17.0	-	8.0	25.0	0.0	Leukaemia
FA3	-	47.7	0.0	2.7	71.0	-	28.0	49.0	0.6	Anaemia
FA4	-	75.7	0.3	0.0	19.7	-	4.0	23.7	0.3	Leukaemia
FA5	-	78.0	0.0	2.7	17.6	-	1.7	19.3	0.0	"
FA6	-	40.7	0.0	3.7	16.0	-	39.3	55.3	0.3	Anaemia
FA7	-	67.0	0.0	0.7	22.3	-	9.0	31.3	1.0	Leukaemia
FA8	-	46.0	0.3	3.0	17.4	-	33.0	50.4	0.3	"
FA9	-	34.7	0.7	1.0	32.0	-	31.6	63.6	0.0	Anaemia
FA10	-	77.6	0.0	2.7	17.0	-	2.7	19.7	0.0	"
FA11	-	45.7	2.0	4.0	24.0	-	24.0	48.0	0.3	Leukaemia
FA12	-	75.0	0.7	0.3	18.0	-	6.0	24.0	0.0	"
FA13	-	81.0	0.3	0.7	14.7	-	3.0	17.7	0.3	"
										HB%
149	9,000	14.0	0.0	20.0	1.0	23.0	40.0	64.0	2.0	55 Chill
150	9,000	14.0	0.0	23.0	3.0	22.5	36.5	62.0	1.0	60 "
151	9,000	52.3	1.0	10.7	8.7	10.7	16.3	35.7	0.3	62 "
171	14,000	68.3	2.7	1.7	7.7	15.3	3.3	26.3	1.0	58 "
172	12,500	56.6	0.4	4.0	7.3	17.0	14.7	39.0	0.0	62 "

DISEASED POULTRY **TABLE** XVII CAECAL COCCIDIOSIS

Ref.	Total WBC's	Differential Count								Haemoglobin %
		Ne	Eo	Ba	Lymphocytes				Mono	
					Large	Medium	Small	Total		
80	-	45.0	1.4	2.0	6.6	12.6	31.7	50.9	0.7	80
140	26,800	16.5	2.25	4.25	9.25	27.25	40.25	76.75	0.25	75
167	28,000	18.3	0.7	2.3	7.7	14.0	57.0	78.7	0.0	65
181	37,000	40.5	3.75	3.75	10.5	11.25	30.25	52.0	0.0	60
194	30,000	49.3	12.0	0.7	23.0	3.0	11.3	37.3	0.7	50
195	21,000	27.7	0.7	4.0	14.0	10.7	36.3	61.0	6.6	70
196	5,000	29.3	0.0	2.0	13.0	23.3	31.7	68.0	0.7	-
206	22,500	43.7	0.0	2.7	8.3	8.7	29.6	46.6	4.0	64
207	9,000	26.3	2.0	1.7	19.7	13.3	34.7	67.7	2.3	60
208	17,000	61.3	1.4	3.7	9.3	8.3	15.0	32.6	1.0	63
209	31,500	70.0	0.0	1.7	8.3	5.7	13.8	27.8	0.5	60
210	22,500	2.5	0.0	3.0	28.0	33.5	30.5	92.0	2.5	62
211	31,000	15.3	0.7	1.3	43.0	13.7	24.0	80.7	2.0	67
247	9,500	31.0	4.3	3.7	12.0	17.3	31.7	61.0	0.0	64
256	27,000	41.3	2.0	4.3	12.3	5.7	32.7	50.7	1.7	80
258	27,000	28.0	0.0	4.7	6.3	30.0	30.3	66.6	0.7	58
259	18,000	34.4	6.3	5.3	5.7	35.0	12.3	53.0	1.0	68
283	45,000	47.0	9.7	4.7	16.7	11.3	10.3	38.3	0.3	45
284	1,800	16.0	0.0	6.0	6.0	4.0	30.0	40.0	38.0	48
293	31,200	38.7	0.0	2.7	6.0	9.0	41.6	56.6	2.0	85

DISEASED POULTRY **TABLE XVIII** DUODENAL COCCIDIOSIS

Ref.	Total WBC's	Differential Count								HB %
		Ne	Eo	Ba	Lymphocytes				Mono	
					Large	Medium	Small	Total		
282	34,000	70.0	0.0	0.0	2.0	20.0	8.0	30.0	0.0	35
212	18,500	23.8	8.5	5.5	14.0	10.8	34.7	59.5	2.8	70
199	38,800	39.7	6.3	1.3	30.0	15.0	8.4	53.4	0.0	78
170	35,800	49.3	2.3	2.3	16.3	9.7	19.7	45.7	0.4	64
145A	30,000	48.0	2.3	1.0	32.7	6.3	9.3	48.3	0.4	55
145B	30,000	47.0	2.0	0.0	27.0	6.0	17.0	50.0	1.0	55
135	1,000	14.0	2.0	34.0	26.0	22.0	2.0	50.0	0.0	55
82	40,000	22.4	3.4	2.4	2.2	15.6	54.0	71.8	0.0	-
81	38,000	23.8	3.1	2.8	2.0	14.4	54.0	70.4	0.0	-
75	-	39.0	3.0	5.0	11.0	4.5	36.5	52.0	1.0	72
74	-	30.0	2.0	3.0	9.5	9.0	45.0	63.5	1.5	70
73	-	36.3	1.0	4.7	13.7	4.6	38.7	57.0	1.0	70

DISEASED POULTRY **TABLE XIX** MISCELLANEOUS

Ref.	Total WBC's	Differential Count								% HB	Condition
		Ne	Eo	Ba	Lymphocytes				Mono		
					Large	Medium	Small	Total			
119	18,500	26.7	2.3	2.3	0.3	4.0	64.4	68.7	0.0	70	Nicotine poisoning
120	9,000	60.0	1.4	2.0	5.3	11.3	19.7	63.3	0.3	100	Peritonitis
136	300	16.0	4.0	48.0	12.0	0.0	20.0	32.0	0.0	55	Intestinal toxaemia
139	16,000	19.7	1.0	2.3	12.3	7.0	56.3	75.6	1.4	80	Perosis
216	54,200	15.7	4.0	1.7	6.0	13.6	57.0	76.6	2.0	70	Arthritis
279	34,000	43.8	2.5	2.2	9.7	8.0	33.5	51.2	0.3	80	Lame
285	22,500	17.7	1.3	1.0	23.0	19.3	36.0	78.3	1.7	75	Intestinal toxaemia
311	28,800	31.3	4.7	1.3	8.3	4.0	50.4	62.7	0.0	64	Nephritis
313	27,500	25.0	7.0	3.0	7.3	15.7	42.0	65.0	0.0	68	Arsenic poisoning
315	27,000	19.3	7.0	3.3	5.7	8.3	56.0	70.0	0.4	70	Arsenic poisoning
344	45,000	15.0	1.6	5.3	7.7	14.7	55.0	77.4	0.7	72	Sick
455	-	14.7	1.3	3.3	0.7	14.0	66.0	80.7	0.0	55	Nephritis

MISCELLANEOUS **TABLE** XX MISCELLANEOUS

Ref.	Total WBC's	Differential Count								
		Ne	Eo	Ba	Lymphocytes				Mono	
					Large	Medium	Small	Total		
NP1	-	20.3	1.3	4.0	11.0	-	62.0	73.0	1.4	Normal Pigeon
NP8	-	27.0	0.0	8.0	-	-	-	65.0	0.0	"
NP30	-	40.0	1.0	4.0	17.0	-	38.0	55.0	0.0	"
171A	14,800	29.0	0.3	0.0	45.7	-	24.0	69.7	1.0	Normal Goat
218A	12,266	36.0	1.5	0.0	5.5	36.0	21.0	62.5	0.0	Normal sheep
126A	26,000	15.7	3.3	0.7	-	-	-	78.3	2.0	"Gid" ewe
169A	22,700	30.3	0.0	0.3	-	-	-	65.7	3.7	Goat kid Rachitis
199A	18,200	40.0	1.0	0.0	3.0	14.0	38.0	55.0	4.0	Rachitic goat
183A	26,000	45.0	5.0	1.0	-	-	-	45.0	4.0	"
192A	53,000	45.0	5.0	1.0	-	-	-	45.0	4.0	Goat - aborted
320	28,800	48.0	1.0	0.3	7.3	25.7	17.7	50.7	0.0	Normal young pigeon
325	38,000	46.0	0.0	1.0	3.3	27.7	21.7	52.7	0.3	Pigeon after heparin

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